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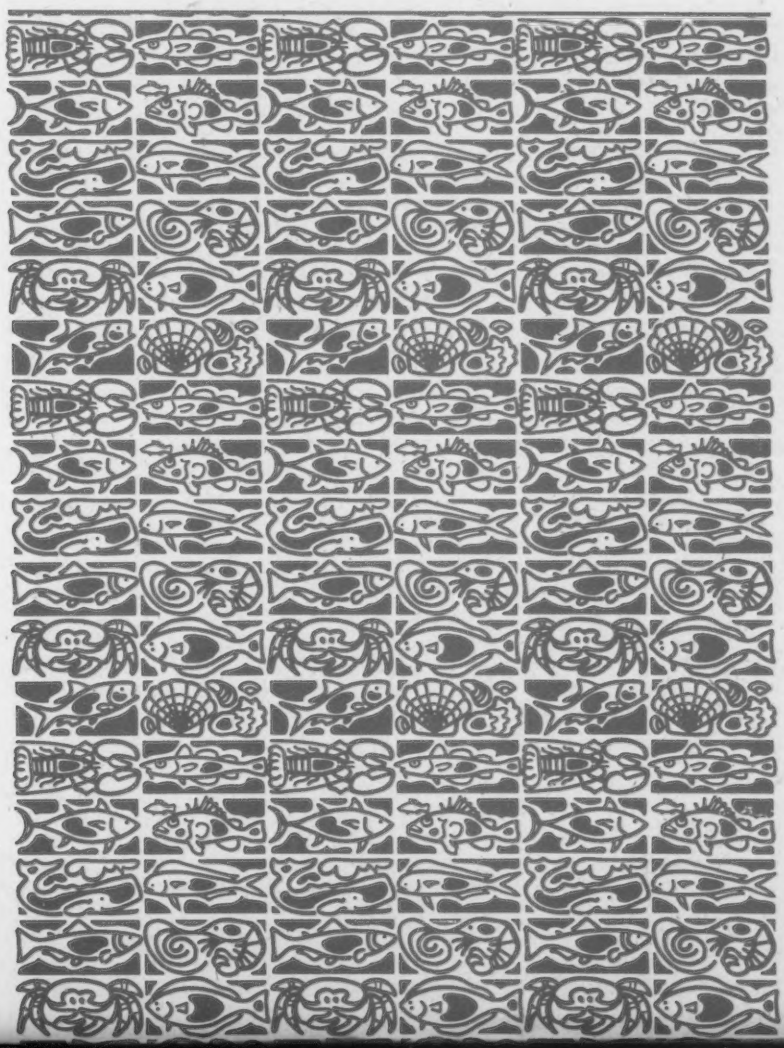

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Optimal sampling for estimating the size structure and mean size of abalone caught in a New South Wales fishery

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Abstract.—The fishery for blacklip abalone, *Haliotis rubra*, is one of the most valuable in New South Wales, Australia. An important part of the stock assessment process for this fishery is to quantify temporal changes in mean size and size structure of abalone in the landed catch. Variation in abalone growth over small spatial scales in this fishery and differences in harvest strategy among different divers result in large variations in sizes of abalone landed. Monte Carlo simulations were used to investigate the influence of these sources of variation on estimates of mean size and size structure. Different sampling scenarios were considered—from random sampling of all diver-days to a more realistic scheme where abalone were subsampled both within and among diver-days. For a given total number of abalone measured, error in estimated mean size and size structure declined asymptotically with increasing numbers of diver-days. By measuring at least 1,500 abalone from 100 diver-days, reliable estimates of size structure and mean size of abalone in the catch for the whole fishery were produced. This conclusion was robust with respect to the number of diver-days in the fishery. Estimated sampling intensity and probabilities of detecting differences based on simulated variances for the whole fishery are provided.

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Sample-size determination remains a crucial exercise in all aspects of ecology and fisheries biology, and the array of analytical tools available continues to grow (e.g. Gerrodette, 1987; Kimura, 1990; Peterman, 1990; Thompson, 1992). The great majority of these techniques are designed to optimize sampling for data derived from independent samples from a number of hierarchical sources of variation (e.g. Schweigert et al., 1985; Sen, 1986; Andrew and Mapstone, 1987; Kitada et al., 1992; Crone, 1995). Methods for determining sample sizes for describing size- or age-frequency distributions are less common (but see Smith and Sedransk, 1982; Schweigert and Sibert, 1983; Parkinson et al., 1988; Erzini, 1990).

Sample-size determination for the simultaneous estimation of different size classes is possible analytically only under limited circumstances in fisheries applications. If differences among individuals are the only source of variation to be contended with, then the proportion of individuals in each size class in a population may be estimated simultaneously by using the methods developed by Fitzpatrick and Scott (1987) and Thompson (1987), and the calculation of the variances of these estimates are simple.

In most situations facing fisheries biologists, however, there are many sources of variation confounding simple random sampling and sample-size determination for estimating mean size at harvest and the underlying size structure. Typically, catches come from many boats, fishermen, and fishing grounds, and samplers are almost always faced with far more fish than they could possibly measure. Under these circumstances there are many sources of variation that may bias sampling. Not least of these is the likelihood of underlying spatial and temporal heterogeneity in the fished populations and changes in fishing behavior. Monte Carlo simulations provide a relatively straightforward, although computation-intensive, means of determining appropriate schemes in these instances. Sample-size determination for multistage survey designs relies on apportioning sampling effort to various levels on the basis of variance or cost (or both).

The fishery and the problem

The fishery for abalone *Haliotis rubra* in New South Wales (NSW) is managed by using a combination of size limits, closures, and output controls. In 1995, each of 37 divers had an annual quota of 9 metric tons (t). Since 1974, divers have

been required to provide details of daily catch weight and diving hours in each of 28 zones (Fig. 1). Divers may catch abalone in any of the zones, which range in length of coastline between 7 and 147 km. In 1994 there was a total of 3,129 diver-days in the fishery and an average of 104 diver-days per zone (Fig. 2A). Based on estimates of average weight per abalone, a catch of between 20 and 760 abalone was landed per diver-day (Fig. 2B). The mean size of abalone caught per diver-day ranged between 116 and 129 mm, although the majority were between 117 and 121 mm long (Fig. 2C). A minimum size limit of 115 mm has been applied to the fishery since 1987. Fishing pressure in this fishery is intense and the size structure of abalone in the landed catch in each zone may be described by negative exponential distributions of varying instantaneous slope (see examples in Fig. 3).

Determining a sampling scheme to provide reliable estimates of the size structure and mean size of abalone in the landed catch is complicated by differences among diver-days. Worthington et al. (1995) and Worthington and Andrew (in press) have described large variations in demographic parameters, such as growth rate, maximum size, mortality, and fecundity, over a range of spatial scales. These studies report as much variation in the rates of growth and in the maximum sizes of abalone within sites separated by 2 km as there was among sites separated by hundreds of kilometers. Sizes of abalone in landed catches will therefore depend on how and where the diver worked as well as on the demographic attributes of the population being fished. For example, on any day, a diver may work areas where abalone are fast-growing and tend to be larger or areas where abalone are slow-growing and smaller (or both).

In this study we report the results of simulations in order to determine an appropriate allocation of sampling effort to estimate mean sizes and size structures of abalone in the landed catch in the New South Wales fishery. Sampling is considered for groups of zones and for the whole fishery. A simulation approach was adopted in preference to an analytical solution (e.g. Cochran, 1977) because we were interested in simultaneously optimizing sampling across a number of size classes—all of which were nonindependent. The simulation procedure allowed an estimation of the deviation of samples of different sizes from a known or true population. Parameters used in the simulations were based on preliminary sampling in 1993–94.

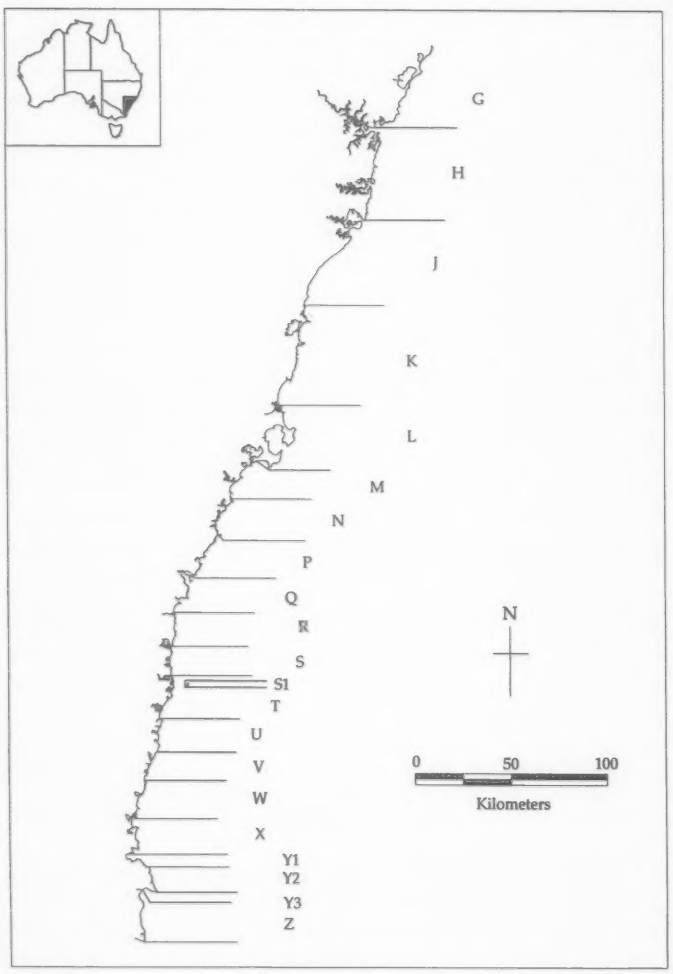

Figure 1

Map of the lower half of New South Wales showing zones in the abalone fishery. The zones are coded alphabetically from north to south.

Materials and methods

Simulation study

A Monte Carlo simulation approach was used to estimate the relative efficiency of three strategies for sampling abalone:

1 Sample all abalone from randomly selected diver-days;
2 Sample a fixed number of abalone randomly from the catches of all diver-days; and

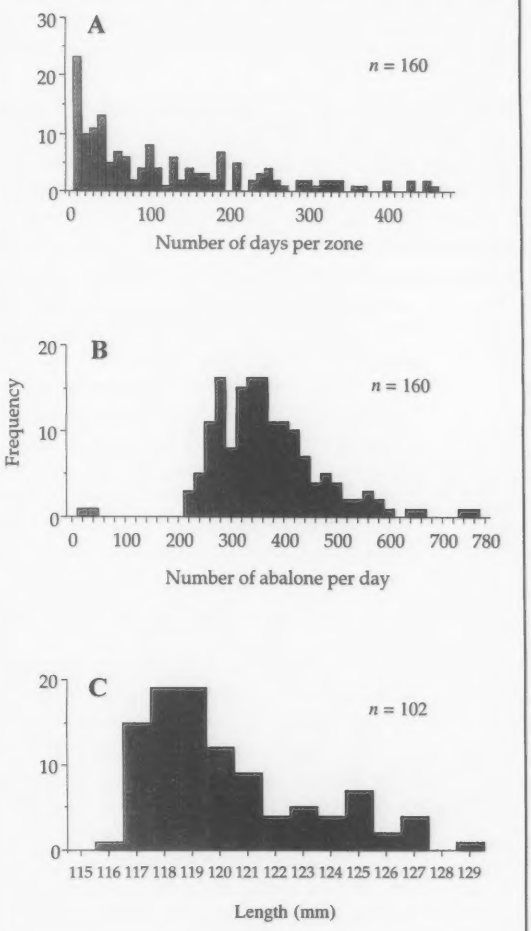

Figure 2

Summary statistics from the NSW abalone fishery for 1993 and 1994: (**A**) Frequency distribution of the number of days per zone, (**B**) Frequency distribution of the number of abalone per diver-day calculated from known average weights per abalone, (**C**) Frequency distribution of the mean length of abalone caught per diver-day. Sample sizes indicated are the number of diver-days.

3 Sample a fixed number of abalone randomly from diver-days selected randomly.

Of the three strategies, the third is the most logistically and financially reasonable. There is considerable unpredictability in where and when divers will work both because of weather conditions and a reluctance by divers to specify where they will work on a given day. These facts conspire to make it difficult to sample in a truly random manner. Nor is it practical to stratify appropriately across either divers or days because the population of diver-days to be sampled can be determined only in retrospect. For these reasons we have used a "diver-day" as the unit of stratification. Three sources of variation are confounded in "diver-day." Differences among divers, as a result of their fishing behavior (e.g. experience and ability) could not be separated from the variation inherent in where they fished, and therefore in the abalone caught. The third source of variation pooled into diver-day is the day itself (e.g. weather and sea conditions). Although these sources of variation were inseparable within the present study, the inferences drawn about a representative sampling scheme are not confounded. Strategy 1, although desirable, would limit the number of diver-days that could be sampled given a fixed total sampling effort. Strategy 2 represents the "ideal" sampling scheme and is used as a standard from which the remaining, more realistic, schemes are judged.

Parameters of the simulation

Parameters were determined for the simulations by using information collected during the 1993–94 fishing years. We assumed that there is as much variability in parameters among diver-days within a zone as among zones. Sampling schemes for zones or groups of zones and for the whole fishery were assessed by varying the total number of diver-days in the "fishery" per year. We therefore ran simulations by using up to 600 diver-days to determine sampling schemes for zones and groups of zones and simulated a 4,000-d fishery to determine a sampling scheme for the fishery as a whole.

Step 1 (determination of the number of abalone caught per diver-day) Based on previous sampling (Fig. 2B), the numbers of abalone caught in all diver-days were grouped into different catch groups ranging from the midpoint of 20 to 760 abalone, with the interval of the catch groups being 20. Thus, the total number of catch groups is 38 (i.e. (760–20)/20 + 1 = 38). The frequency of the number of abalone caught per diver-day was then estimated. Based on these frequencies, the total catch per diver-day was determined by multinominal sampling described as follows. Let P_J = probability of the number of abalone harvested in a diver-day in catch group J, where J = 1, 2, ..., 38. The catch of diver-day i was determined by generating a random number R between 0 and 1 based on the uniform distribution and by assigning this number to one of the catch groups. The catch was assigned to catch group J if the random number followed

$$\sum_{k=1}^{J-1} P_k \le R < \sum_{k=1}^{J} P_k .$$

After determining the catch group (i.e. J) for abalone harvested in diver-day i, the number of abalone harvested in diver-day i was determined as

$$C_i = (J - 0.5)20 + 20U,$$

where U is a random number between 0 and 1 generated from a uniform distribution. Because J in this equation has been determined from the previous equation, we have omitted the subscript $_J$ from C_i for the sake of simplicity. This procedure was repeated for all diver-days in each simulation to determine the number of abalone harvested in each diver-day.

Step 2 (determination of the mean length of abalone caught per diver-day) The mean length of the catch for each diver-day was determined from the estimates derived from sampling 102 diver-days in 1993–94 (Fig. 2C). Lengths of abalone were measured to the nearest mm from catches from a range of zones and are assumed to be measured without error. The estimates of mean size ranged from 116 to 129 mm. Let P_I = probability of the mean length in length interval I (I = 1, 2, ..., 14). The mean length for diver-day i was determined by generating a random number R between 0 and 1 based on a uniform distribution and by assigning this number to one of the length intervals. The length was assigned to length interval I if the random number followed

$$\sum_{k=1}^{j-1} P_k \le R < \sum_{k=1}^{j} P_k .$$

After determining the length interval (I) for the mean length of abalone harvested in diver-day i, the mean length of diver-day i was determined as

$$L_i = I + 115(\text{mm}),$$

where 115 mm is the size limit. This procedure was repeated for all diver-days in each simulation to determine the mean length of abalone caught per diver-day.

Step 3 (determination of length composition of abalone caught per diver-day) The size distributions of abalone caught in a diver-day may be described by exponential distributions with varying slope, truncated at the lower limit by the legal size limit and at the upper limit by the value T, which was determined by random draws from the range of extreme values observed in preliminary sampling. The density function for such an exponential distribution can be written as

$$P(x) = Ae^{\frac{-(x-a)}{\sigma}},$$

where $a \le x \le T$, and A and σ are to be estimated. For an exponential distribution

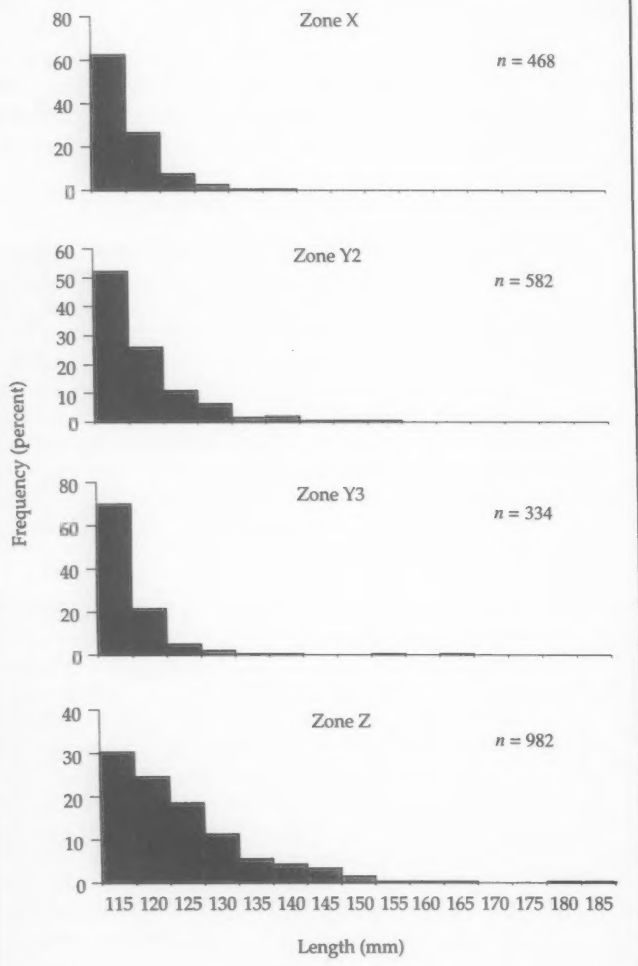

Figure 3

Examples of length-frequency distributions of abalone from four zones in the NSW abalone fishery. Data were pooled among diver-days from 1994.

with the above density function, $E(x) = a + \sigma$ and A is a constant defined by a, σ, and T. For a discrete variable X with the interval width d, constant A can be approximately estimated from

$$A\sum_{i=1}^{N} e^{-\frac{(x_i-a)}{\sigma}} = 1,$$

where

$$N = \frac{T-a}{d} + 1.$$

Solving this equation, we have

$$A = \frac{1}{\sum_{i=1}^{N} e^{-\frac{(x_i-a)}{\sigma}}}.$$

The lower bound (parameter a) is the size limit of 115 mm and is the same for all diver-days. Parameter T ranges from 117 to 150 mm among diver-days. For a diver-day i, parameter T_i was determined by randomly drawing an integer between 117 and 150 mm on the basis of the uniform distribution. For diver-day i, the mean size s_i was randomly selected from the frequency distribution of mean size estimated from data gathered in 1993–94 (Fig. 2C). The error σ for diver-day i is estimated as $\sigma_i = s_i - a$. Thus, the probability of abalone in length interval j being caught on diver-day i, $P^*_{i,j}$, was calculated. The number of abalone in length interval j caught in diver-day i, $C_{i,j}$, was then calculated as

$$C_{i,j} = C_i P^*_{i,j}.$$

Evaluation of the simulation

The size-frequency distribution and mean size of abalone at harvest calculated from the total catch of all diver-days were used as the "true" population of landed catch. Different sample sizes were used for each sampling scenario and compared to this known population. For each sampling scenario, 100 simulation runs were conducted. An error index, after measuring the difference between length-frequency distributions calculated from the catch of all diver-days in a year and from the sampled catch, was calculated as

$$\textit{Error index} = \frac{\sum_{h=1}^{100}\sqrt{\sum_{k=1}^{N}(T_k - O_{h,k})^2}}{100},$$

where T_k is the frequency of abalone in length interval k calculated from the total catch of all diver-days. This population was fixed among runs h calculated from the total catch of all diver-days, and $O_{h,k}$ is the frequency of abalone in length interval k in simulation run h calculated from the sampled catch. Thus the error index provides an index of the summed deviations from the true population across all size classes.

The difference between mean sizes of abalone estimated from the catch of all diver-days and from the sampled catch was evaluated by using an index defined as

$$\textit{Average absolute difference in mean} = \frac{\sum_{h=1}^{100}\left|\hat{M}_h - M\right|}{100},$$

where $\hat{M}_h$ is the estimated mean of the h^{th} simulation run for the sample catch and M is the mean of the total catch (i.e. the true mean size). The distribution of the calculated difference in means for 100 simulation runs was used to evaluate the variation in estimated average difference in mean size.

Results

We concentrated on results of simulations appropriate to estimating the size structure and mean size of abalone for the smaller spatial scale—that of zones or groups of zones. Results will be presented across a range of sample sizes for zones or groups of zones with up to 600 diver-days per year. We briefly discuss results for the whole fishery by sampling 100 diver-days in a fishery of 4,000 diver-days under scenario 3. Probabilities of detecting changes in mean size of abalone under this scheme are provided.

Under scenario 1, in which all abalone caught in a randomly selected diver-day are measured, there was a sharp decline in the error index of size composition as the number of diver-days sampled increased from 1 to 10 (Fig. 4). After 10 diver-days, the rate of decline in the error index slowed markedly. As expected, as the number of diver-days sampled approached the number of diver-days in the fishery, the error approached zero (Fig. 4). There was little variation in the error index among fisheries with 400 to 600 diver-days per year (Fig. 4). A similar pattern was observed in comparing differences in the mean size between the sampled catch and the total population for all sizes of the fishery (Fig. 4). For example, irrespective of the number of diver-days per year, when 10 diver-days were sampled, the average difference in mean size was 5 mm over 100 simulation runs.

Under scenario 2, diver-days were ignored as a source of variation and all abalone caught during the year had an equal probability of being sampled. This

is not a reasonable sampling scheme for most fisheries but provides a standard against which the others may be judged. Many more abalone must be measured under scenario 1 than scenario 2 to achieve comparable error indices (Figs. 4 and 5). For example, if under scenario 1 a total of 10 diver-days are sampled (approximately 3,400 abalone measured), then the error index is approximately 0.04. This level of error could be achieved by measuring only 400 abalone randomly distributed across all diver-days. As an example of variation in sampling under scenario 2, consider the differences in error index and difference in mean size when sampling 100 abalone and 1,000 abalone in a fishery of 400 diver-days (Fig. 5). Across the range considered (100–600 diver-days), the size of the fishery made little difference to estimates of error in the size composition or mean size of individuals (Fig. 5).

Under scenario 3, the most realistic of the sampling schemes, there were large differences in the error index, depending both on the number of abalone sampled in total and the number of diver-days sampled (Fig. 6). The magnitude of error was not, however, greatly influenced by the total number of diver-days per year (Fig. 6). The results suggest that, although the error in estimating the size-frequency composition depended on the total number of abalone sampled, the rate of decline in the error index was similar among all sample sizes (Fig. 6). In all cases, the rate of decline in the error index reached an asymptote at approximately 20 diver-days. For zones or groups of zones with up to 600 diver-days per year, there was an approximate two-fold reduction in the error index by increasing the total number of abalone measured from 100 to 1,000 abalone (Fig. 6). There was little further reduction in the error index by increasing replication from 1,000 to 1,500 abalone (Fig. 6).

The average error in estimated mean size of abalone declined rapidly with increasing number of diver-days between 1 and 15 diver-days (Fig. 7). Further increases in simulated sampling effort produced relatively modest gains without large increases in the number of diver-days sampled. For example, doubling the sampling effort from 15 to 30 diver-days caused only minor increases in precision (Fig. 7). In contrast to the patterns in errors in estimated size composition, increases in the total number of abalone measured from 100 to 1,500 produced little reduction in the average difference in mean size (Fig. 7).

The relative importance of diver-days as a source of variation is demonstrated by the difference between sampling a total of 100 abalone spread across five days and sampling 2 abalone on each of 50 diver-days (Fig. 7). In the latter case, the average difference in mean size between the true and estimated distributions and the sample was approximately 2 mm, in contrast to 6 mm when 100 abalone were sampled in 5 diver-days. The difference between observed and expected means was considerably smaller in comparing 200 abalone in each of 5 diver-days with the same total number of abalone spread over 50 diver-days irrespective of the size of the fishery (Fig. 7).

In considering the sampling scheme required for the whole fishery, scenario

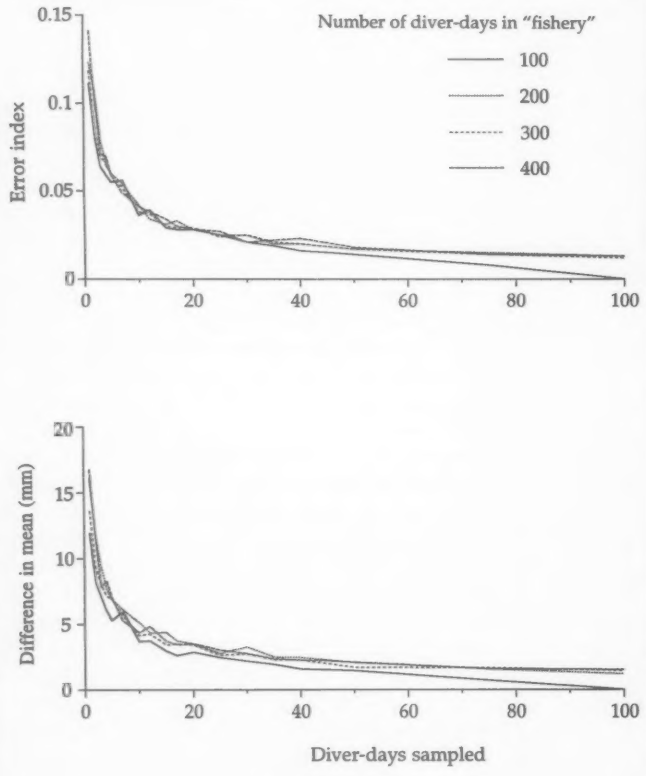

Figure 4

Errors in size composition and estimated mean size of abalone caught in different sizes of the "fishery" with sampling scenario 1. Calculation of the error index for the size composition and of the average difference in mean is described in the text.

3 was scaled up to a larger number of diver-days per year. In 1994, there was a total of 3,129 diver-days in the fishery. We simulated the efficiency of measuring 50 abalone per day in 100 diver-days in a fishery of 4,000 diver-days. At this sampling intensity, the estimated error index for the size structure and average difference in estimated mean size of abalone were 0.018 and 1.5 mm respectively.

The cumulative frequency distribution (Fig. 8) presents the probability of correctly rejecting the hypothesis of no difference under two scenarios. In the first, the probability of detecting a "real" difference between an estimated mean size and a nominated size is given. This nominated size may be a management benchmark, significant deviation from which will cause a change in management, such as a quota reduction. If, for example, the difference between a management threshold and an estimated mean size is 3 mm, there is an 85% chance that the observed difference is "real" and not sampling error (Fig. 8, line b). If the observed difference is greater than 3 mm, then the probability of this being due to sampling error is less than 15%.

In the second scenario, this logic is extended to situations in which differences among years are considered. In this instance, both estimates of mean size are measured with error. If, for example, there is a 3-mm difference in mean size between two years, the probability of incorrectly interpreting this as a real difference among years, i.e. more than sampling error, is $0.85 \times 0.85 = 0.72$ (Fig. 8, line a).

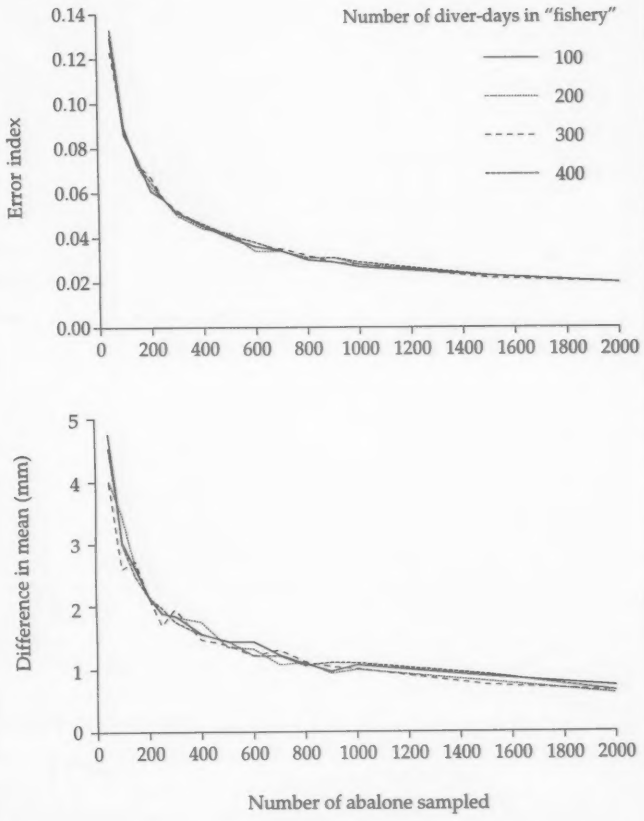

Figure 5

Errors in size composition and estimated mean size of abalone caught in different sizes of the "fishery" with sampling scenario 2.

Discussion

Many stock assessment methods rely on reconstructions of the demography of exploited populations, using age-structured information (e.g. Fournier and Archibald, 1982; Deriso et al., 1985; Megrey, 1989; Kimura, 1990; Terceiro et al., 1992). Stock assessment methods available for species that cannot be aged are more limited, although recent development in size-based analogues of age-structured models have expanded the range of methods available (e.g. Sullivan et al., 1990). Size-based methods have traditionally relied on reducing size-frequency distributions into cohorts (e.g. Bhattacharya, 1967; Schnute and Fournier, 1980; Grant et al., 1987; Castro and Erzini, 1988). The reliability of these methods depends in large part on the representativeness of the sample distributions and the shape of the size-frequency distribution (e.g. Smith and Maguire, 1983; Chen, 1996).

The sampling scheme described in this study is essentially a stratified random design, with diver-day being the intermediate stratified factor (see also Sen, 1986; Kitada et al., 1992; Crone, 1995). An alternative approach used in sample-size determination for estimating age composition has been described by Schweigert and Sibert (1983). Their approach was to determine sample-size requirements for each size and age class separately and to develop an overall sampling scheme as a compromise solu-

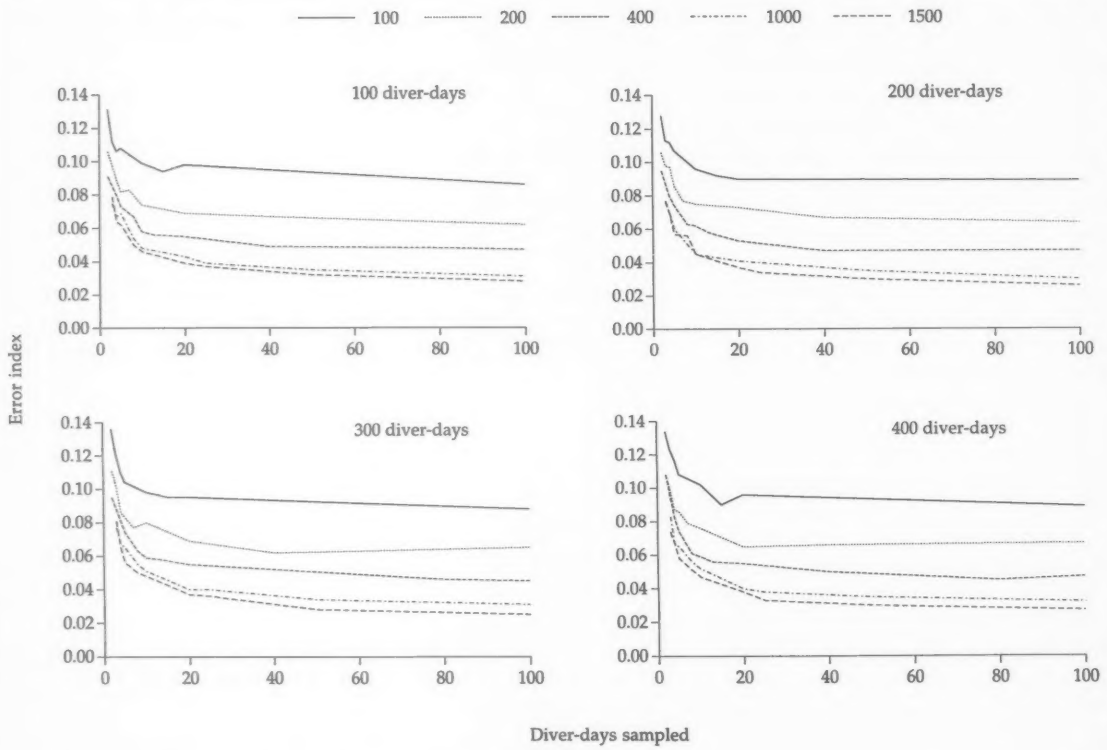

Figure 6

Errors in estimating the size composition of abalone caught in different sizes of the "fishery" with sampling scenario 3. "Fisheries" of 100, 200, 300, and 400 diver-days were considered. Sample sizes indicated on the figure are the total number of abalone measured.

tion among those classes (see also Horppila and Peltonen, 1992). This approach was not appropriate for our situation because we were interested only in the size structure of landed abalone—*Haliotis rubra* can not be reliably aged by analysis of growth rings (McShane and Smith, 1992). The Monte Carlo approach that was adopted allowed us to simulate the simultaneous estimation of all size classes in the population.

The simulations described were based on the assumption that the size structures of abalone within a diver-day were distributed as a negative exponential function. If the fishery declined, the mean size of abalone would probably decline and the slope of the fitted exponential curve would increase. If this occurred, then the sampling scheme described would be conservative. If, however, the fishery improved and larger abalone were caught, then the size-frequency distribution might depart from the negative exponential distribution. If such a departure was significant, then the simulations would need to be reparameterized and each sampling scenario reexamined to determine an appropriate sample size for estimating the size structure of abalone in catches.

One of the objectives of this simulation study was to estimate the probability of detecting varying changes in mean size of abalone at harvest. Advice on trends in mean size at harvest may be given at two spatial scales: that of the whole fishery and that for individual zones or groups of adjacent zones. At present, the NSW abalone fishery is managed as one stock— size limit regulations and quota allocations are made on a statewide basis. Management measures, such as closures, are, however, possible at the smaller spatial scale if there are declines in indices of abundance (including mean size at harvest). Indeed, abalone stocks are increasingly being seen as comprising metapopulations of relatively discrete populations (see references in Shepherd et al., 1992), and thus such management measures are likely to be effective.

The simulations suggest that sampling more than 1,500 abalone spread across 100 diver-days would

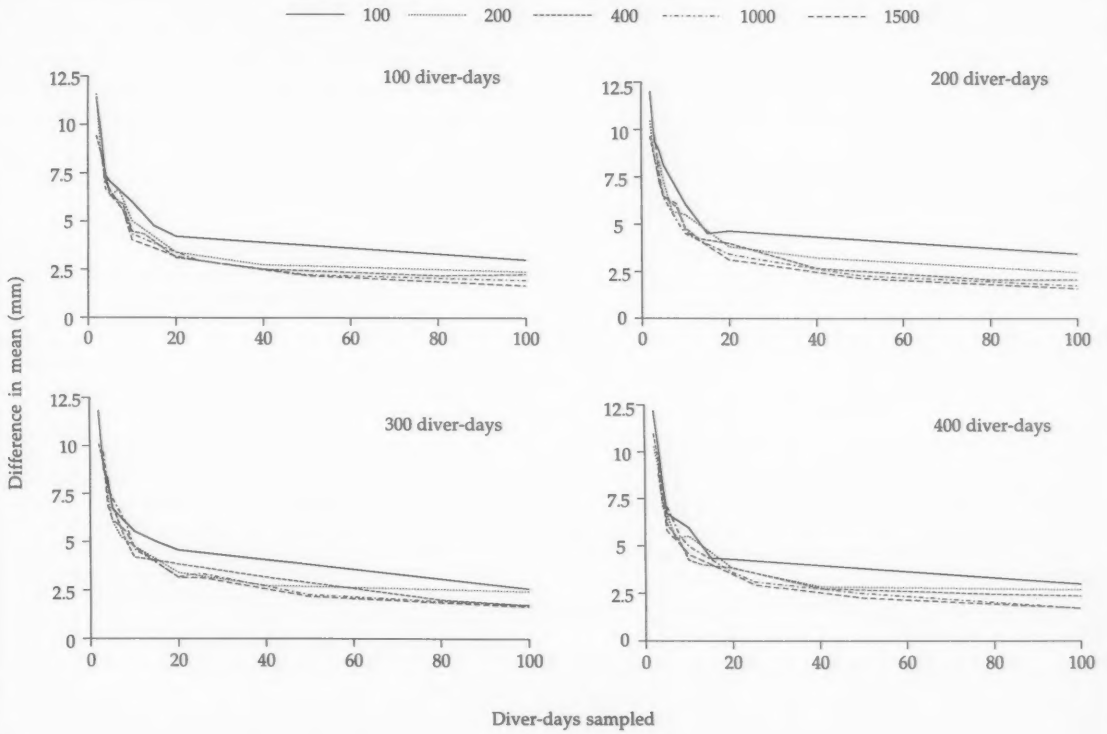

Figure 7

Errors in estimating mean size of abalone caught in different sizes of the "fishery" using sampling scenario 3. "Fisheries" of 100, 200, 300, and 400 diver-days were considered. Sample sizes indicated on the figure are the total number of abalone measured.

provide reliable estimates of the size structure of abalone in the landed catch and would detect relatively small changes in the mean size of abalone at harvest for the whole fishery. How small those changes are will be determined by how likely managers are prepared to accept the possibility of being wrong. At a smaller scale, 1,000 abalone from 20 diver-days would be needed to achieve a similar degree of discrimination. More intensive sampling did not greatly improve the reliability of these estimates over the range simulated. The simulations suggest that differences among diver-days were an important source of variation (see also Kitada et al., 1992; Crone, 1995).

Interpretation of trends in mean size of individuals among years requires some care. Divers may change their diving patterns among years, and fish populations may change with different demographic attributes. These patterns alone may produce changes in the composition of the landed catch independent of any underlying trend in the fishery. In essence, this problem is analogous to that in interpreting catch-rate information from heterogenous fisheries, such as abalone. Several authors have claimed that apparent stability of catch rate is possible despite declining abundance of abalone as a result of changing diver behavior (e.g. Hilborn and Walters, 1987).

In these simulations we did not weight sampling effort in scenario 3 for the number of abalone caught in a diver-day. We, therefore, assume that there is no relation between the size and number of abalone caught. Using data from 1993–94, we found that there is no significant relation between the number of abalone caught in a diver-day and the size of those abalone. The impact of weighting is further diminished by the fact that the fraction sampled per day is relatively high, irrespective of the number caught (usually >25%). We assume that this sampling fraction provides a reliable estimate of mean size within diver-days.

The results of these simulations suggest that the large sample sizes possible in estimating mean size

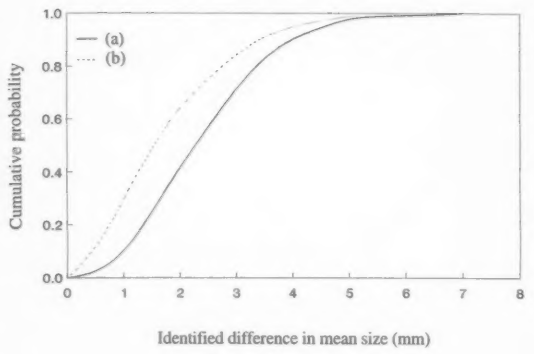

Figure 8

Cumulative distribution of the probability of correctly rejecting the null hypothesis of no difference between (**A**) two estimates, both measured with error and (**B**) an estimated mean size and a predefined value, measured without error. The distribution is based on 1,000 simulation runs with a sampling intensity of 50 abalone per day in 100 diver-days in a fishery of 4,000 diver-days.

of abalone at harvest may provide a false sense of reliability if higher level sources of variation are ignored. If variation above that found among abalone within a diver-day is not included, false conclusions may be drawn about the statistical significance of trends through time, both because of unrepresentative sampling and imprecise estimates of variability.

In the present study, the relatively large differences in sizes of abalone among diver-days meant that even relatively high sampling fractions (>10 %) would have a relatively low probability of detecting changes in mean size less than 3 mm. This conclusion appeared to be robust over a realistic range in the number of diver-days sampled. Given the broad similarities between the sampling scheme described for this fishery and those in many commercial fisheries, concerns may be raised about the reliability of samples taken from processing plants in the absence of an understanding of the contributions of higher level sources of variation. The simulation framework described may be directly expanded to accommodate more complex situations.

Acknowledgments

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Seasonal and ontogenetic changes in distribution and abundance of smooth flounder, *Pleuronectes putnami*, and winter flounder, *Pleuronectes americanus*, along estuarine depth and salinity gradients

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Abstract.—The distribution and abundance of two potentially competing flatfish species, smooth flounder, *Pleuronectes putnami*, and winter flounder, *Pleuronectes americanus*, were examined along salinity and depth gradients in upper Great Bay Estuary, New Hampshire. Both species were abundant in the estuary but exhibited differential use of habitats along both gradients. Smooth flounder were most abundant at the mesohaline, riverine habitat, whereas winter flounder were most abundant at the polyhaline, open-bay habitat. Both species exhibited a generalized up-river movement as salinity increased with the seasons. Smooth flounder showed ontogenetic changes in distribution along the depth gradient, with smallest individuals occupying shallowest depths. Intertidal mudflats were an important nursery area for young-of-the-year smooth flounder. Winter flounder showed little separation by size along the depth gradient, and few were found in the intertidal mudflat habitat. The potential for competition between these two species is lessened by their partial segregation along the gradients examined.

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Smooth flounder, *Pleuronectes putnami*, and winter flounder, *Pleuronectes americanus*, are dominant members of fish communities in estuaries along the east coast of North America, co-occurring from Newfoundland, Canada, to Massachusetts Bay, USA. These morphologically similar species are sympatric over much of their geographic ranges. However, little is known of their spatial overlap within specific estuaries. Winter flounder use estuaries primarily as nursery grounds, whereas adults spend most of their lives in coastal waters (Bigelow and Schroeder, 1953; Pearcy, 1962; Scott and Scott, 1988). In contrast, smooth flounder complete their entire life cycle within estuaries.

Smooth flounder prefer softer bottom substrata than winter flounder (Bigelow and Schroeder, 1953), and Jackson (1922) noted they were most abundant in the low-salinity regions within Great Bay Estuary, New Hampshire. Little else is known of their intraestuarine habitat preferences. Several studies have examined movements and habitat use of juvenile winter flounder in estuaries south of Cape Cod (e.g. Pearcy, 1962; Saucerman 1991). However, because many northern estuaries differ considerably from those south of Cape Cod, most obviously in their temperature regimes, it is possible that juvenile winter flounder use northern estuaries differently from ones to the south, as has been shown to be the case for adults (Hanson and Courtenay, 1996).

The purpose of this study was to provide a quantitative comparison of the occurrence of smooth and winter flounder in various habitats in upper Great Bay Estuary, New Hampshire. The habitats comprised gradients defined by depth or salinity. Comparative studies along habitat gradients can define which habitats are important to a species, especially in relation to different life history stages; such analyses can also be used to study the relative importance of physical and biotic factors in limiting species distributions (Connor and Bowers, 1987). Examination of the shape of species-abundance curves along a gradient can provide inferences into whether competition or physiological limitations are important in setting dis-

tributions (Terborgh, 1971) and can lead to the generation of testable hypotheses.

Methods

Study area

Great Bay Estuary (Fig. 1) is a complex embayment comprising the Piscataqua River, Little Bay, and Great Bay. It is a tidally dominated system and is at the confluence of seven major rivers and several small creeks, as well as the water from the Gulf of Maine (Short, 1992). Great Bay Estuary is a drowned river valley, with high tidal energy and deep channels with fringing mud flats. The main habitat types within the estuary are mudflat, eelgrass, salt marsh, channel bottom, and rocky intertidal. This study was conducted in the upper estuary, referred to as Great Bay, although preliminary sampling took place in the lower estuary also. Great Bay is a large, shallow embayment having an average depth of 2.7 m, with deeper channels extending to 17.7 m (Short, 1992) and a tidal range of about 2 m. The water surface of Great Bay covers 23 km^2 at mean high water and 11 km^2 at mean low water (Turgeon, 1976). Greater than 50% of the sediment surface of Great Bay is exposed mud or eelgrass flat at low tide. The Squamscott and Lamprey Rivers are major sources of freshwater to Great Bay. River flow varies considerably on a seasonal basis but is generally highest during spring runoff. Vertical stratification of Great Bay is rare because of strong tide- and wind-induced currents, although partial stratification may occur during periods of high freshwater runoff, particularly at the upper tidal reaches of rivers (Short, 1992).

Smooth and winter flounder were sampled monthly, May 1989 through September 1991, at five sites in upper Great Bay Estuary (Fig. 1). Ice cover prevented sampling from December through March in all study years. A 4.8-m otter trawl of 38-mm stretch mesh, with a 25-mm stretch mesh codend and a 6-mm codend liner, was used for sampling. Preliminary studies indicated that the net retained flounder as small as 25-mm total length (TL). A sample consisted of all flounder collected in one 10-minute tow at approximately 2.5 knots. Four samples were taken at each site from April to November. Two samples were taken within two hours (±) of low slack tide, one tow with the tidal current and one tow against, and two samples were taken similarly around high slack tide. All flounder collected were measured to the nearest mm TL. Bottom temperature and salinity were measured after each tow with a Beckman Model 510 temperature, conductivity, and salinity meter.

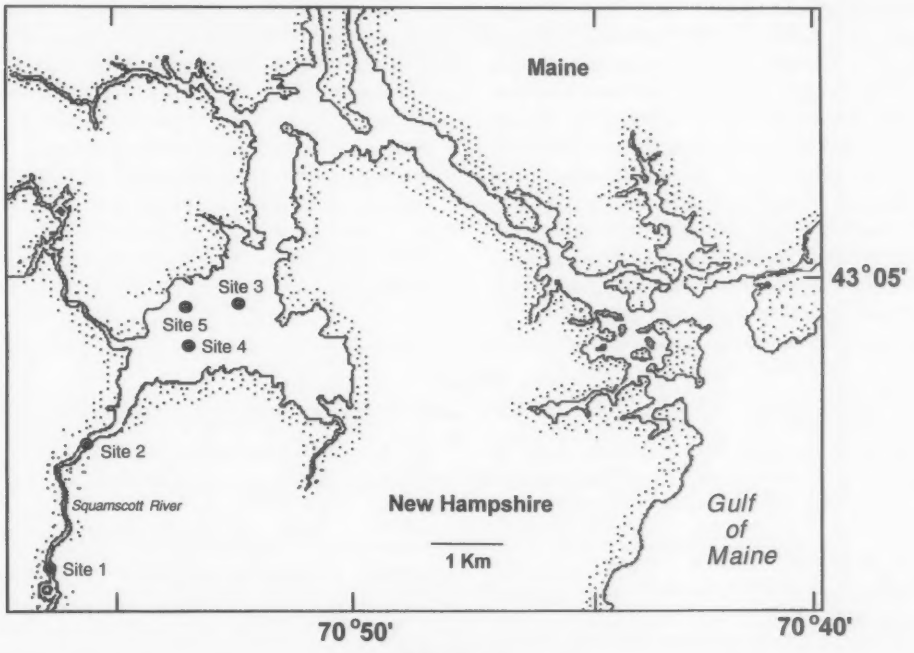

Figure 1

Study area. Survey sites were all located in Great Bay Estuary, New Hampshire, as indicated.

Site 1 (low salinity, Squamscott River at Route 51), site 2 (medium salinity, Squamscott River at Route 108), and site 3 (high salinity, middle of Great Bay) were located along a salinity gradient formed by Great Bay Estuary and one of its major tributaries (Fig. 1). The mean salinity value at each site varied considerably on a seasonal basis, but a salinity gradient always persisted along these sites. Table 1 summarizes some physical characteristics of these locations. Site 1 was located in the Squamscott River about 4 km above the mouth. Although the river is still tidal in this area, the water is often fresh or extremely low in salinity. Site 2 is also located in the Squamscott River but only 0.5 km above the mouth. Salinity at this site is highly variable but intermediate between the other two sites. Site 3, the site with greatest salinity, was located in the middle of Great Bay proper. The depth and bottom substratum were similar at all three stations (Table 1).

Sites 3 and 4 (high salinity, shallow Great Bay) and site 5 (high salinity, Great Bay intertidal flats) were located along a depth gradient in a contiguous area in the middle of Great Bay. Site 3 was the deepest site sampled along the depth gradient. Site 4 represented the intermediate depth, and site 5 was located on intertidal mudflats and therefore sampled only on high tides. All three sites had similar bottom substratum, silty mud, and owing to their proximity, experienced nearly identical salinities (Table 1).

Monthly length frequencies at each site were pooled over all study years. The Kolmogorov-Smirnov test was used to test for differences in length-frequency distributions among sites. One-way analysis of variance (ANOVA) was used to test for significant differences in catches among the three sites that made up each of the two gradients. To reduce the number of ANOVA's performed and to increase the power of the tests by increasing sample sizes, the monthly data were grouped into three seasons: spring, summer, and autumn. Months of April, May, and June were considered spring; July and August were considered summer; and September, October, and November were considered autumn. Because many months contained zero catches and, in some cases, the variances were proportionate to the means, the data were transformed by using a square-root transformation (square root(X+1)). The Kolmogorov-Smirnov test with the Lilliefors modification and probability plots of residuals indicated no significant deviations from normality, and Levene's test indicated homogeneity of variances after the transformation. Where a significant difference in catches was detected among sites, the sites were compared by using Tukey's HSD test (Zar, 1984).

Results

A total of 8,333 smooth flounder and 2,105 winter flounder were captured during the study period. Both juvenile and adult smooth flounder were abundant in the study area in contrast to winter flounder, which were abundant only as juveniles. However, length frequencies of the two flounders were similar because adult smooth flounder are about the same size as juvenile winter flounder. Smooth flounder were captured from many different year classes, whereas winter flounder were primarily age 0^+, 1^+, and 2^+, based on length frequencies.

Salinity followed a typical boreal estuarine seasonal pattern (Figs. 2 and 3). The general trend at all stations was for salinity to be lowest in April, to increase over the late spring and summer months reaching the highest levels during August and September, and to decline during autumn. These seasonal patterns were especially pronounced at site 1 and site 2. Salinities in spring of 1991 were higher at all sites than in the other two years, a result of an uncharacteristically dry spring and limited spring runoff. Another salinity anomaly occurring in 1991

Table 1

Physical characteristics of the sampling sites. Sites 1, 2, and 3 make up the salinity gradient, whereas sites 4, 5, and 3 form the depth gradient.

Site number and habitat type	Salinity (ppt)		Temperature (°C)		Depth (m)		
	Mean	Range	Mean	Range	Mean	Range	Bottom type
1 (low salinity)	4.2	0.0–22.4	19.4	4.7–25.7	2.7	1.9–4.0	silty mud
2 (medium salinity)	10.9	0.4–24.0	17.1	0.0–27.8	3.7	1.8–4.3	silty mud
3 (high salinity, greatest depth)	20.3	6.5–29.9	15.4	1.8–23.9	6.2	4.9–7.9	silty mud
4 (intermediate depth)	20.9	6.5–29.5	16.4	2.3–24.9	2.1	1.5–4.4	silty mud
5 (intertidal flats)	19.8	11.0–28.5	15.2	0.2–24.2	1.5	1.1–2.2	silty mud

was a sudden decrease in salinity in September caused by dilution from the heavy rains with Hurricane Bob in late August of that year. Sites comprising the depth gradient had similar patterns of salinity in all years of the study.

Salinity gradient

Both species were unevenly distributed along the salinity gradient, and their distributions changed seasonally (Table 2). The timing of peak abundance of smooth flounder at site 1 varied from year to year. In 1989 and 1990 smooth flounder were abundant in mid to late summer (Fig. 4). The influx of smooth flounder was associated with seasonal changes in the salinity regime from fresh to oligohaline (Fig. 2). In 1991, smooth flounder were present at site 1 in all months sampled. In this year, salinity was higher than that during the two previous years (Fig. 2). Length frequencies of smooth flounder at site 1 (Fig. 5) were significantly different ($P<0.0001$ in all monthly K-S tests, May–October) from those at site 2 (Fig. 6), although the difference appears to be less in the autumn than in the spring. Larger fish (>100 mm) made up a higher proportion of the catch at site 1 in comparison with site 2, indicating differential migration among size classes. Winter flounder were rarely collected at site 1 (Fig. 7). They were found there only on a few occasions in September and October when salinity was at a seasonal high.

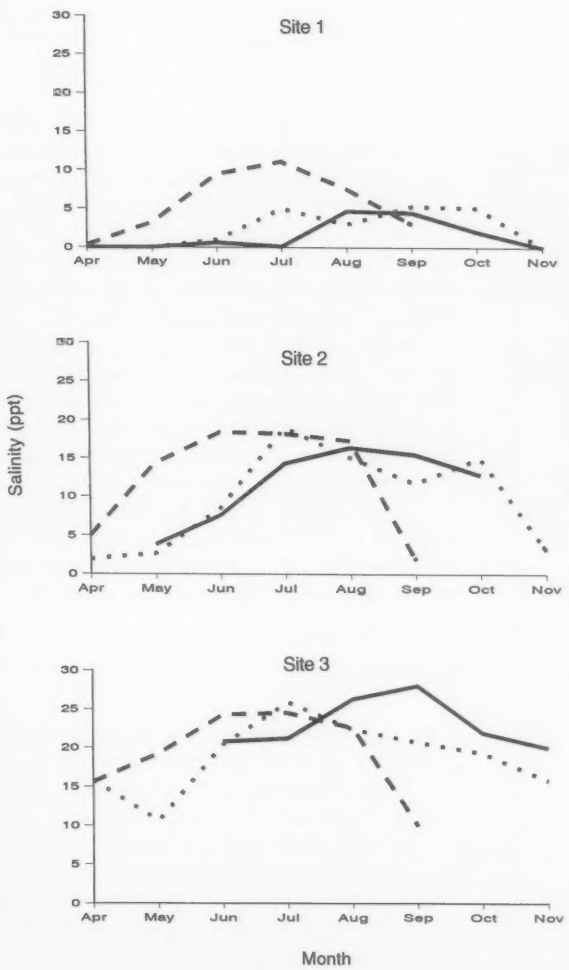

Figure 2

Salinity at three sites sampled along a salinity gradient in Great Bay Estuary, New Hampshire. Mean salinity was highest at site 3 (20.3 ppt) followed by site 2 (10.9 ppt) and then site 1 (4.2 ppt). Solid line = 1989; dotted line = 1990; dashed line = 1991.

Smooth flounder were abundant at site 2 during all months, and their average abundance at this site exceeded that of all other sites. Their abundance was generally high in the spring, lower in late summer to early autumn, and high again in mid to late autumn (Fig. 4). This trend was opposite to that observed for site 1. Correlation analysis of catches of smooth flounder at sites 1 and 2 indicated a weak but significant negative relationship ($P=0.032$, $r=-0.48$). When catches were large at site 1, they tended to be small at site 2. This finding suggests that the same population of smooth flounder was migrating between the stations, although the length frequencies show that a greater proportion of larger smooth flounder than smaller smooth flounder travel the 3 km between the sites.

Winter flounder were abundant at site 2 only during autumn (Fig. 7), although even during these periods of abundance, the catches of winter flounder were always lower than those for smooth flounder. The movement of winter flounder into site 2 from Great Bay proper was associated with relatively high salinities (Fig. 2) and with low abundances of smooth flounder (Fig. 4). The length frequencies of winter flounder collected from site 2 (Fig. 6) and site 3 (Fig. 8) were similar.

Smooth flounder occurred at site 3 in relative abundance only in April, May, and June (Fig. 4). Catches of smooth flounder decreased significantly after June in all years. Winter flounder were most abundant at this site than at the other two sites comprising the salinity

gradient (Fig. 7). They were present in relatively large numbers during all months. There were no significant differences in catches of winter flounder among months for all study years.

Depth gradient

The two species of flounder showed a differential use of the three sites that comprised the depth gradient. There were also differences in the sizes of flounder that used the three sites. Seasonal changes in distribution were less pronounced than those exhibited along the salinity gradient.

A broad size range of smooth flounder used site 3 (Fig. 8). However, their abundance dropped off sharply after June of each year, as previously discussed (Fig. 9). Winter flounder showed few seasonal trends in abundance at this station (Fig. 10). A broad size range of juvenile winter flounder was found here. A distinct influx of young-of-the-year winter flounder could be seen at site 3 from August through November of each year (Fig. 8).

At site 4, smooth flounder showed little seasonal change in abundance (Fig. 9), although there was a trend for catches to be lowest in late summer and early autumn. Length frequencies differed between site 3 and site 4. At site 4, few larger smooth flounder were present during any season (Fig. 11), whereas young-of-the-year, which were absent from site 3, were collected at most times. Abundance of winter flounder at site 4 was lowest in all years in early summer (Fig. 10), and catches were always smaller than those at site 3. Length frequencies indicated that smaller winter flounder made up a greater proportion of the catch at site 4 (Fig. 11) as compared to site 3 (Fig. 8).

Catches at site 5 were very variable for both species and showed no clear seasonal patterns (Figs. 9 and 10). Smooth flounder catches at site 5 were dominated by young-of-the-year. Few larger (>100 mm TL) individuals were ever caught at this site (Fig. 12), in contrast to site 3 (Fig. 8) but similar to site 4 (Fig. 11). Winter flounder occurred at site 5 sporadically and in very low numbers (Fig. 10). Catches of winter flounder were a mix of different sizes of juveniles.

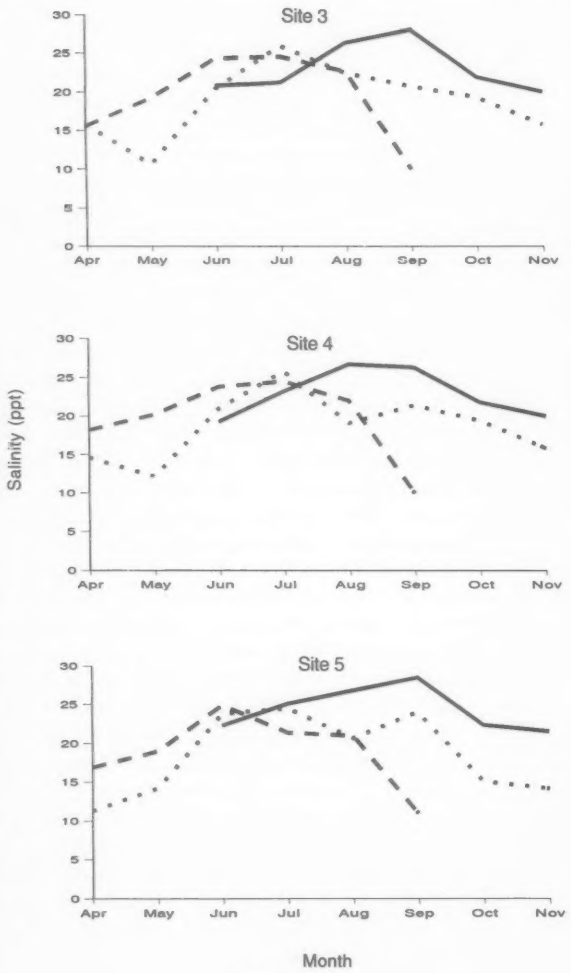

Figure 3

Salinity at three sites sampled along a depth gradient in Great Bay Estuary, New Hampshire. Mean depth was greatest at site 3 (mean = 6.2 m) followed by site 4 (2.1 m) and then site 5 (1.5 m). Solid line = 1989; dotted line = 1990; dashed line = 1991.

Discussion

A variety of habitats are available to smooth and winter flounder in upper Great Bay Estuary. It was the purpose of this study to quantify the occurrence of these two species in various habitats. Although the species ranges of smooth and winter flounder overlap broadly, the evidence presented here indicates that they use habitats within the estuaries differently and that their habitat use is subject to seasonal variations.

Salinity gradient

In general, smooth flounder were most abundant at site 2, the mesohaline river mouth

Table 2

Results of ANOVA's testing for differences in catches of smooth and winter flounder among three sites along the salinity gradient (sites 1, 2, and 3) and three sites along the depth gradient (5, 4, and 3). If there was a significant difference ($P<0.05$) in catches among sites, the results of Tukey's HSD test are listed from lowest to highest. See Table 1 for a description of the sites. ns = not significant.

Year and season	Smooth flounder		Winter flounder	
	Salinity gradient	(*F*-value; df)	Salinity gradient	(*F*-value; df)
1989				
Spring	site 1<site 3<site 2	(25.80; 2,20)	site 1=site 2<site 3	(46.64; 2,20)
Summer	site 3<site 1=site 2	(6.13; 2,27)	site 1=site 2<site 3	(38.11; 2,27)
Autumn	site 3<site 1=site 2	(20.29; 2,23)	site 1<site 3<site 2	(12.62; 2,27)
1990				
Spring	site 3=site 1<site 2	(36.42; 2,22)	site 1=site 2<site 3	(8.24; 2,22)
Summer	site 3<site 1=site 2	(13.88; 2,20)	site 1<site 2=site 3	(12.99; 2,20)
Autumn	site 3<site 2=site 1	(9.69; 2,23)	ns	(0.56; 2,23)
1991				
Spring	site 3<site 2=site 1	(9.52; 2,29)	site 1=site 2<site 3	(11.56; 2,29)
Summer	site 3<site 2=site 1	(4.80; 2,21)	site 1=site 2<site 3	(7.07; 2,21)
Autumn	site 3<site 1<site 2	(121.79; 2,5)	site 1<site 3<site 2	(21.26; 2,5)

Year and season	Smooth flounder		Winter flounder	
	Depth gradient	(*F*-value; df)	Depth gradient	(*F*-value: df)
1989				
Spring	ns	(2.53; 2,11)	site 5<site 4<site 3	(17.15; 2,11)
Summer	ns	(0.28; 2,21)	site 4=site 5<site 3	(16.62; 2,21)
Autumn	site 3=site 5<site 4	(3.73; 2,23)	site 5<site 4=site 3	(7.02; 2,29)
1990				
Spring	ns	(0.13; 2,28)	site 5<site 4=site 3	(10.87; 2,28)
Summer	site 3<site 5=site 4	(4.41; 2,20)	site 5=site 4<site 3	(14.03; 2,20)
Autumn	site 3=site 4<site 5	(5.63; 2,29)	site 5=site 4<site 3	(7.96; 2,29)
1991				
Spring	ns	(1.08; 2,29)	site 5=site 4<site 3	(13.45; 2,29)
Summer	site 3=site 4<site 5	(14.19; 2,17)	site 5=site 4<site 3	(5.87; 2,17)
Autumn	site 3=site 4<site 5	(162.99; 2,5)	site 5=site 4<site 3	(5.82; 2,5)

habitat. Seasonal movements were seen into and out of the oligohaline riverine station (site 1) and the polyhaline station in Great Bay proper (site 3). In all years, there was an up-estuary movement of smooth flounder associated with increasing salinity in summer and early autumn. This movement was most pronounced for larger smooth flounder. Greater movement by larger individuals is probably related to their superior locomotive abilities due simply to their larger body size. This trend towards increasing range of movement with increasing body size has also been found in the hogchoker, *Trinectes maculatus*, a flatfish that is similar in general size to smooth flounders and that is also found in estuarine rivers (Dovel et al., 1969; Smith, 1986).

There is little information available on the distribution of smooth flounder along salinity gradients. Targett and McCleave (1974) found smooth flounder to be abundant in the Sheepscott River–Back Bay River estuary, Maine, in salinities of 17.3–24.7 ppt. Fried (1973), studying the same estuary, found that smooth flounder were not present above 28.5 ppt, whereas winter flounder occurred throughout the salinity range sampled (12.5 to 32.5 ppt). Gordon and Dadswell (1984) found the greatest abundance of smooth flounder in "warm, turbid, low-salinity water" in the upper reaches of the Bay of Fundy. Smooth flounder larvae were most abundant in the low-salinity portion of the St. Lawrence River estuary (Powles et al., 1984). The conclusion of the present

study, that the center of greatest abundance for smooth flounder is in the mesohaline part of the estuary, is in agreement with these previous studies.

Site 3 was the site of greatest abundance for winter flounder. Movements into site 2 were seen in late summer or early autumn in all years. Little information is available concerning the response of juvenile winter flounder to salinity gradients. Most studies on the distribution of winter flounder have considered only temperature or light as important abiotic factors that influence seasonal or short-term movements (McCracken, 1963; Oviatt and Nixon, 1973, Casterlin and Reynolds, 1982). Pearcy (1962) found a relatively homogeneous distribution of age-1 winter flounder throughout a salinity gradient in Mystic River Estuary, Connecticut, that was maintained through all seasons. However, his lowest salinity station was higher in salinity than both site 1 and site 2; therefore he did not sample habitats that might be only seasonably available. Pearcy (1962) also documented movement of young-of-the-year winter flounder from the lower estuary to the upper estuary during the summer months. Indirect evidence for similar movement by young-of-the-year winter flounder in Great Bay Estuary is presented here. No young-of-the-year winter flounder were caught in upper Great Bay until late summer and early autumn (Figs. 8 and 11), indicating an influx from the lower estuary. The lack of small winter flounder during the early part of the year was not an artifact of gear selectivity because young-of-the-year smooth flounder as small as 25 mm TL were caught, indicating that small young-of-the-year winter flounder would have been caught also if they had been present. Winter flounder spawn in the middle and lower portions of Great Bay Estuary and adjacent to the estuary in shallow coastal waters. Young-of-the-year winter flounder show little movement for a few months after metamorphosis (Saucerman, 1991); therefore it is not until they reach a larger size (30–50 mm TL) that they begin to move into the upper estuary.

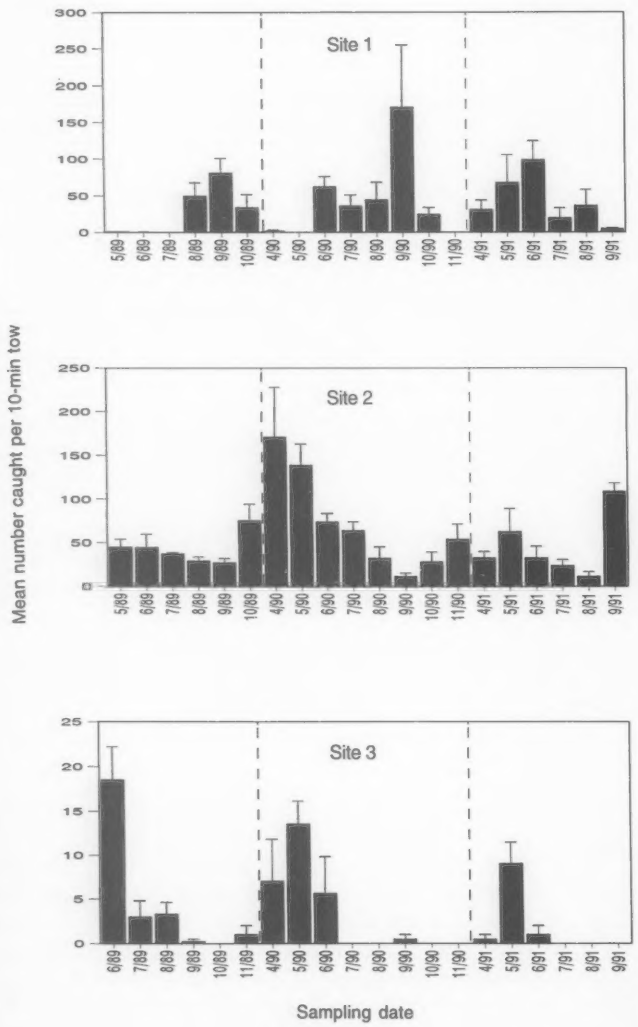

Figure 4

Mean number of smooth flounder, *Pleuronectes putnami*, caught per ten minute tow at three sites along a salinity gradient in Great Bay Estuary, New Hampshire, May 1989–September 1991. Site 1 = oligohaline; site 2 = mesohaline; site 3 = polyhaline. Error bars are one standard error of the mean.

Salinity is considered one of the most important factors affecting habitat use by estuarine fishes. The distributions and movements of several flatfish species including *Solea solea* (Coggan and Dando, 1988; Dorel et al., 1991), *Pleuronectes platessa* (Poxton and Nasir, 1985), and *Platichthys flesus* (Riley et al., 1981; Kerstan, 1991) have been correlated with salinity. A natural estuarine salinity gradient, in which habitats are categorized from benign to harsh in relation to tolerance by species, may serve as part of a continuum of physiological stress (Peterson and Ross, 1991). Species seeking to maximize growth must

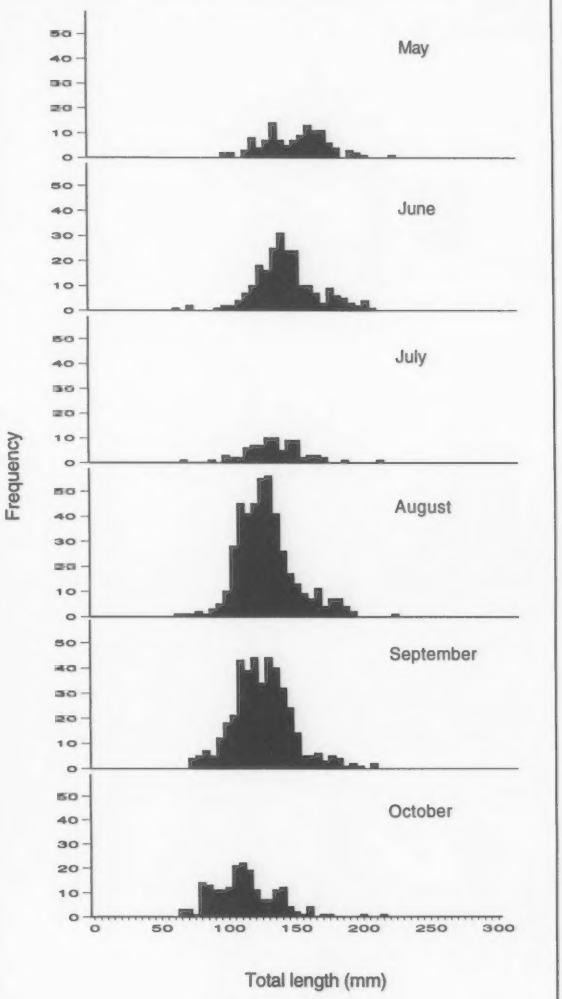

Figure 5

Monthly length frequencies (5-mm size classes) of smooth flounder, *Pleuronectes putnami*, at site 1, pooled over 1989–91.

choose habitats that are of least cost bioenergetically. Several estuarine fish species have been found to be most abundant along a salinity gradient where their metabolic costs of osmoregulation were minimal, including *Ambassis* spp. (Martin, 1990), *Leiostomus xanthurus* and *Micropogonias undulatus* (Moser and Gerry, 1989), and *Paralichthys* spp. (Peters, 1971). Conversely, Peters and Boyd (1972) found that hogchokers, *Trinectes maculatus*, underwent movements that appeared physiologically disadvantageous. They concluded that other factors, in addition to salinity, must be considered. Salinity may provide a broad abiotic framework (Menge and Olson, 1990) within which biotic interactions, such as competition, predation, and prey abundance, can act to modify distributions.

Depth gradient

Smooth flounder showed clear segregation by size along the depth gradient. Larger (>100 mm TL) smooth flounder occurred primarily at the deep-water station (site 3). They were abundant only during April–June, before migrating upriver as salinity increased. Small numbers remained at site 4 throughout the summer and autumn. The tidal flats (site 5) and shallow bay (site 4) were important nursery areas for smooth flounder. Young-of-the-year smooth flounder did not show a dramatic decrease in abundance during the summer, as seen in the larger individuals, and did not appear to make a pronounced seasonal up-estuary movement. Their inferior swimming ability, compared with that of larger individuals, or their inability to osmoregulate efficiently in lower salinity areas may underlie their relatively stationary habits. The tendency for smooth flounder to segregate by size, with the smaller individuals occurring in the intertidal and shallow subtidal areas, has been found in several other flatfish species including English sole, *Parophrys vetulus* (Toole, 1980), and European plaice, *Pleuronectes platessa* (Gibson, 1973; Kuipers, 1973). Segregation by size may reduce intraspecific competition. The intertidal zone may also function as a refuge from predators for small flatfish or as an abundant source of appropriate-size prey items (Toole, 1980). Ruiz et al. (1993) found that shallow water functioned as a refuge from size-selective predation on juveniles of several species of fish and crustaceans in Chesapeake Bay. Van der Veer and Bergmann (1986) found that young-of-the-year European plaice used tidal flats as a refuge from predators rather than for feeding purposes. Potential predators on smooth flounder in Great Bay Estuary include sand shrimp (*Crangon septemspinosus*), grubbies (*Myoxocephalus aeneus*), bluefish (*Pomatomus saltatrix*), striped bass (*Morone saxatilis*), white perch (*Morone americanus*), great blue heron (*Ardea herodias*), and double-crested cormorants (*Phalacrocorax auritus*). Predation by large piscine predators is probably reduced in shallow water, and avian predation is likely increased. Sand shrimp were abundant in trawl samples from both channel and flats areas. The value of tidal flats as refugia from predation cannot be assessed without knowledge of the relative rates of predation by these different predatory groups.

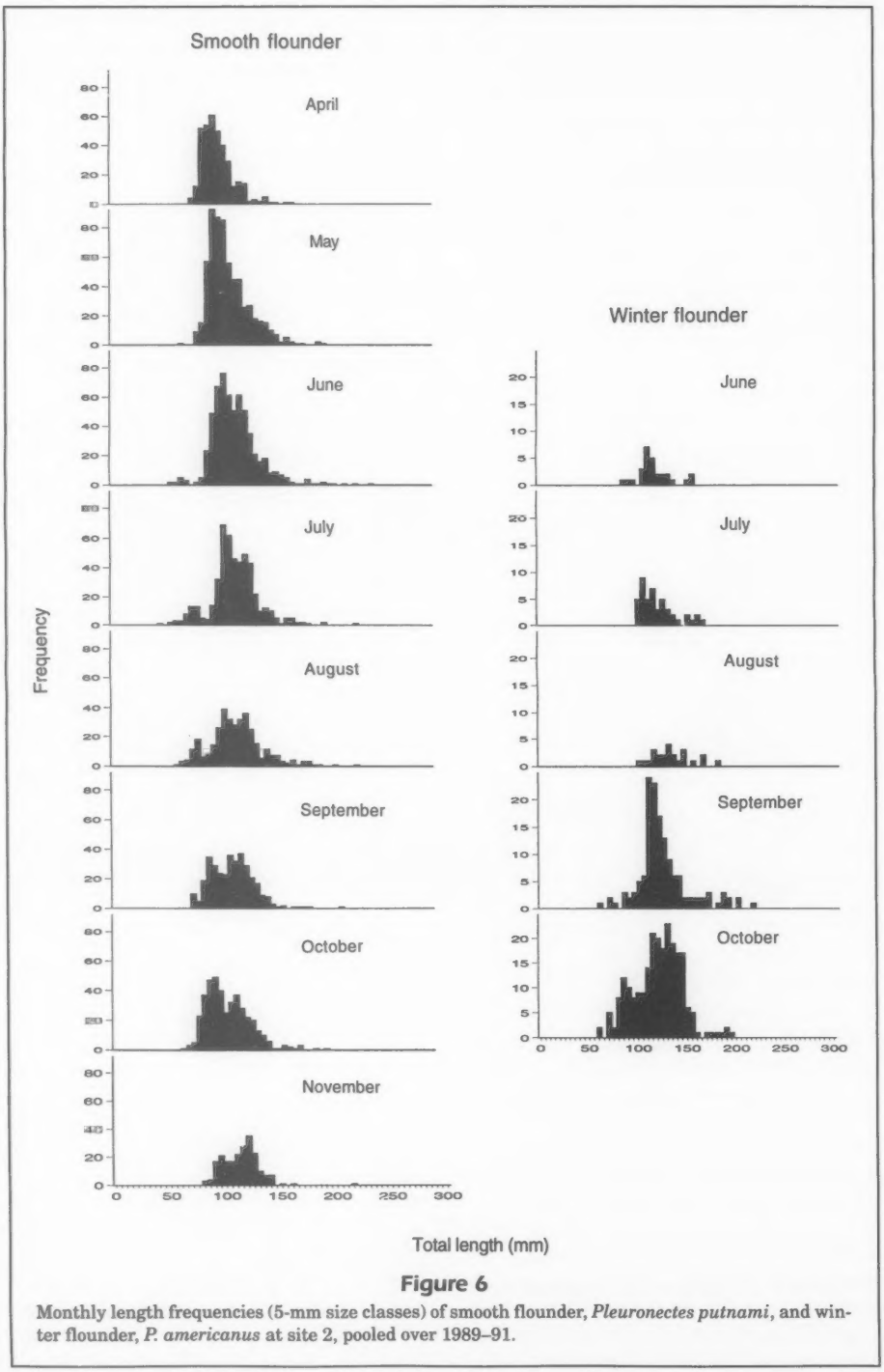

Figure 6

Monthly length frequencies (5-mm size classes) of smooth flounder, *Pleuronectes putnami*, and winter flounder, *P. americanus* at site 2, pooled over 1989–91.

Winter flounder showed little segregation by size along the depth gradient. This finding is in contrast with other studies, which have shown that juvenile winter flounder segregate by size along depth gradients according to differential preferences to temperature and light intensity, with smallest individuals found

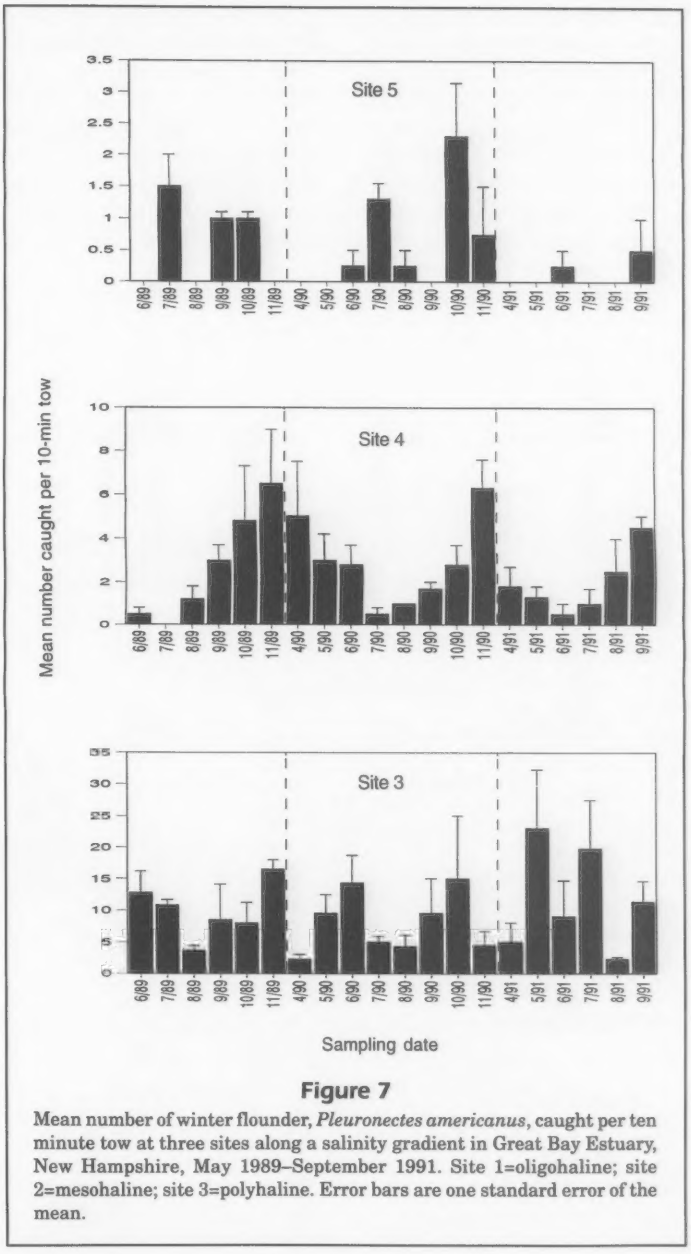

Figure 7

Mean number of winter flounder, *Pleuronectes americanus*, caught per ten minute tow at three sites along a salinity gradient in Great Bay Estuary, New Hampshire, May 1989–September 1991. Site 1=oligohaline; site 2=mesohaline; site 3=polyhaline. Error bars are one standard error of the mean.

at higher temperatures and light intensities (see reviews in Klein-MacPhee, 1978; Casterlin and Reynolds, 1982). It is especially interesting that winter flounder showed relatively little use of the intertidal flats. Tyler (1971), Wells et al. (1973), and Black and Miller (1991), however, found that winter flounder used intertidal flats extensively. Their studies took place in areas of higher salinity where no smooth flounder occurred. Their finding suggests that competition with smooth flounder may be a possible reason for the near absence of winter flounder from the intertidal flats habitat in Great Bay. Targett and McCleave (1974) found that the tidal mudflats in Montsweag Bay, Maine, were dominated by smooth flounder, whereas Fried (1973) found that the channel areas in the same estuary were dominated by winter flounder. Fried (1973) felt that the tidal mudflats offered smooth flounders a refugium from competition with winter flounder and that winter floun-

der were unable to use this habitat type for reasons other than competition with smooth flounder.

Temperature may be a factor in the winter flounder's avoidance of tidal mudflats. Hoff and Westman

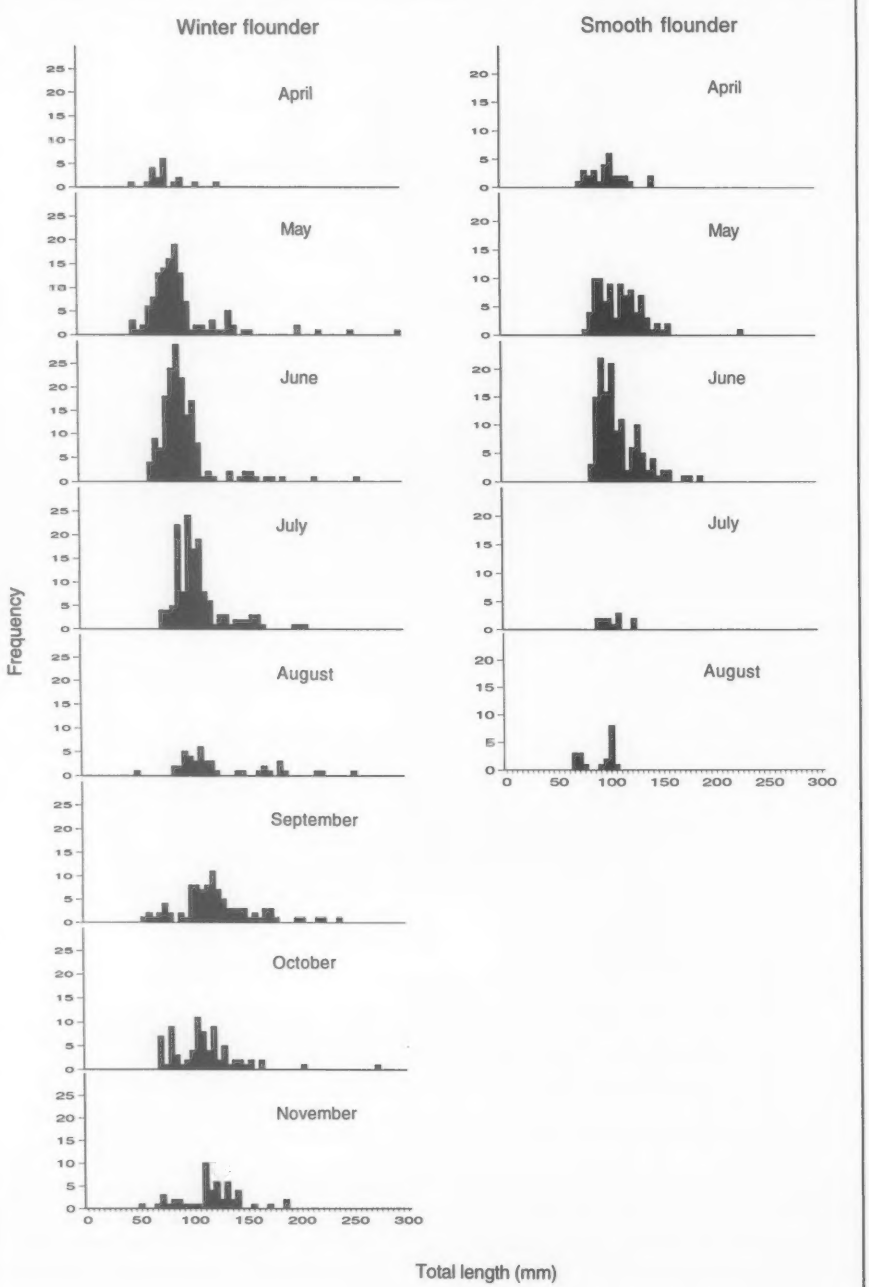

Figure 8

Monthly length frequencies (5-mm size classes) of smooth flounder, *Pleuronectes putnami*, and winter flounder, *P. americanus*, at site 3, pooled over 1989–91.

(1966) found that winter flounder, acclimated to 21°C, had an upper lethal temperature of 27°C. Pearcy (1962) found an upper lethal temperature of 30°C for flounder collected during the summer in Mystic River Estuary. Olla et al. (1969) observed that winter flounder exposed to temperatures above 22.2°C. buried themselves in sediment and ceased to feed. Although comparable data do not exist for smooth flounder, Huntsman and Sparks (1924) reported that upper lethal temperatures for smooth flounder were 2–4°C higher than those for winter flounder. In Great Bay Estuary, temperature may be a factor in determining the relative distribution of the two species in late summer when water temperatures at site 5 reached 22–24.2°C but would not be a factor during most of the year. The low abundance of winter flounder at site 5 persisted during times of the year when temperature would not seem to be limiting.

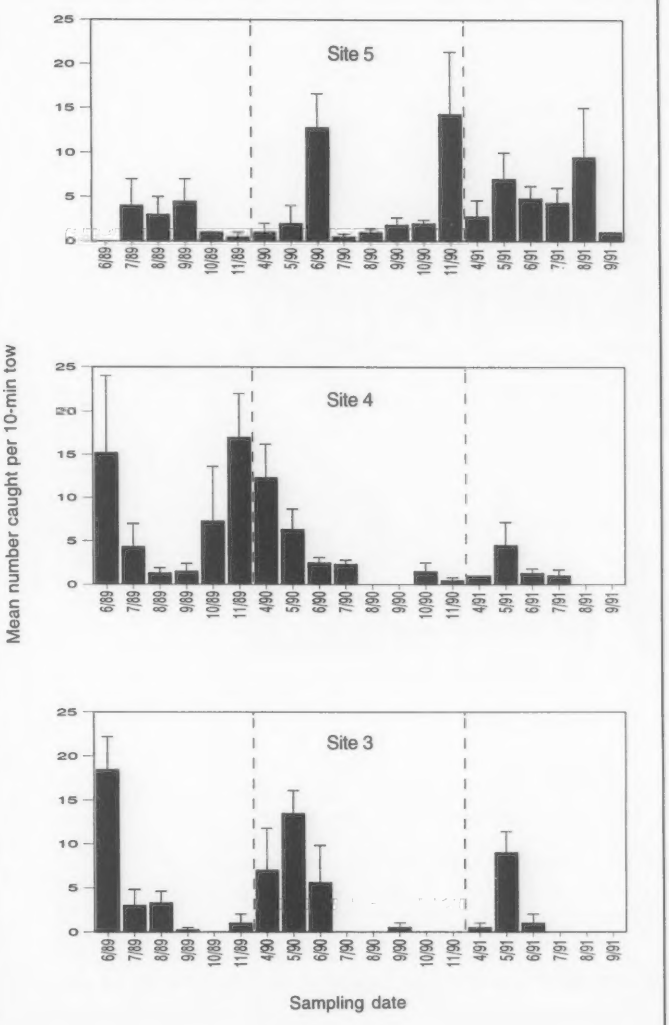

Figure 9

Mean number of smooth flounder, *Pleuronectes putnami*, caught per ten minute tow at three sites along a depth gradient in Great Bay Estuary, New Hampshire. Mean depth was greatest at site 3 (mean=6.2 m) followed by site 4 (2.1 m) and then site 5 (1.5 m). Error bars are one standard error of the mean.

Subtrate preference may play a role in excluding winter flounder from intertidal flats in Great Bay Estuary. Although the bottom type appeared similar (silty mud) at all three sites along the depth gradient (Table 1), this similarity was based on gross examination of core samples. No detailed sediment size analysis was conducted for this study (nor in Fried [1973] or Targett and McCleave [1974]), and therefore differences in sediment structure may have been present between sites but not noted on a gross scale. Sogard (1992) found that growth of winter flounder was negatively correlated with percent silt; faster growth occurred in sandier sediments. Bigelow and Schroeder (1953) found that winter flounder were more abundant on coarser sediments, in comparison with smooth flounder which were more abundant in muddier sediments. Thus, if the channel areas of Great Bay Estuary have coarser sediments than the intertidal flats, perhaps the coarser sediment may explain the difference in distribution along the depth gradient.

Summary

Smooth and winter flounder are partially segregated as species along salinity and depth gradients in upper Great Bay Estuary. It appears that this is due to differential responses to the physical and chemical regime, but the effects of seasonal changes in biotic interactions cannot be excluded. Smooth and winter flounder feed on similar prey items in Great Bay Estuary (Laszlo, 1972; Armstrong, 1995). Competition or movements related to prey abundance may influence their respective distributions. There are many instances where com-

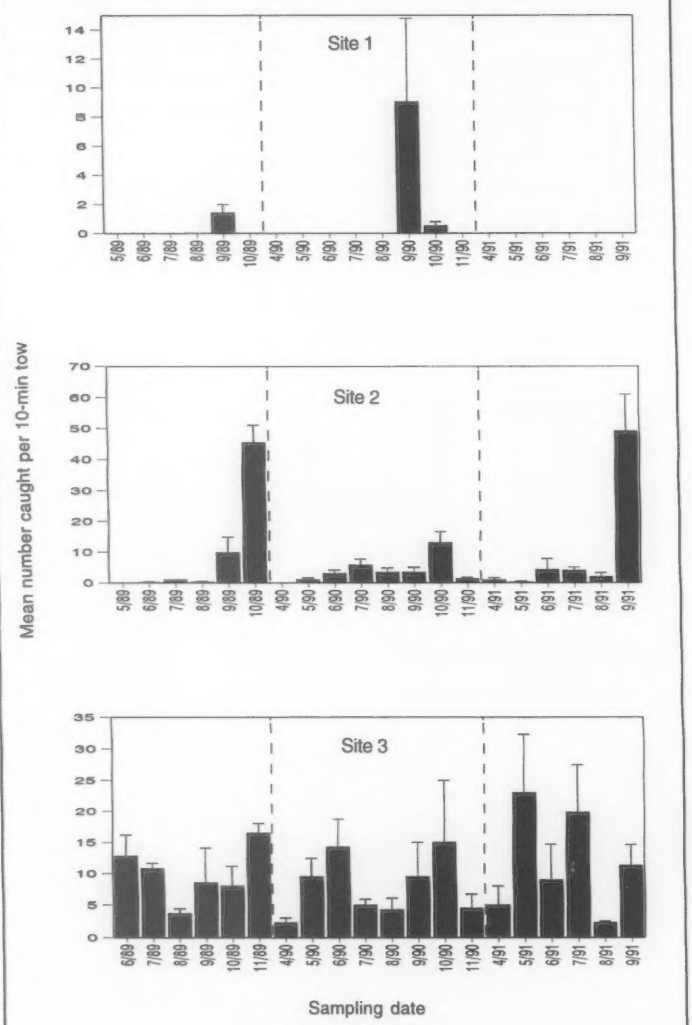

Figure 10

Mean number of winter flounder, *Pleuronectes americanus*, caught per ten minute tow at three sites along a depth gradient in Great Bay Estuary, New Hampshire. Mean depth was greatest at site 3 (mean=6.2 m) followed by site 4 (2.1 m) and then site 5 (1.5 m). Error bars are one standard error of the mean.

petition appears to play a role in the distribution of ecologically similar species along environmental gradients (Connor and Bowers, 1987). In Great Bay Estuary, low salinity and intertidal flats appear to provide at least a partial refugium for smooth flounder from competition with winter flounder.

The relation between smooth and winter flounder changes on a seasonal basis. At times their segregation on a spatial scale is nearly complete, whereas at other times, particularly April–June at site 3 and September–October at site 2, they overlap considerably. Competition theory predicts that niches should vary temporally as a function of resource abundance and of the population densities of potential competitors (Llewellyn and Jenkins, 1987). The predominant temporal pattern of niche overlap seen in studies is increased overlap during resource abundance (Schoener, 1982; Ross, 1986). The periods of great-

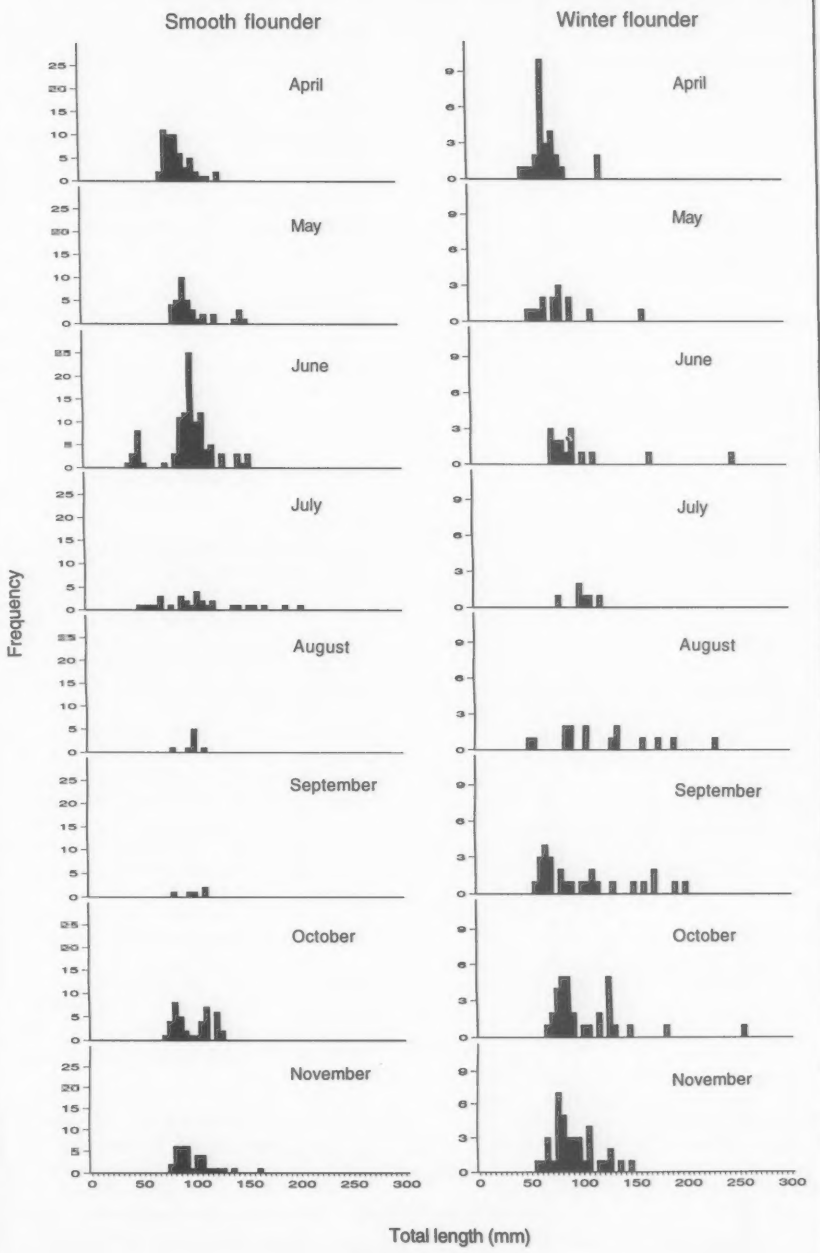

Figure 11

Monthly length frequencies (5-mm size classes) of smooth flounder, *Pleuronectes putnami*, and winter flounder, *P. americanus*, at site 4, pooled over 1989–91.

est overlap in habitat use seen for smooth and winter flounder may be associated with an abundance of some resource, for example, a shared prey item(s).

The upper Great Bay Estuary is an important area for both species. This study has shown the dynamic nature of habitat use by smooth and winter floun-

der. Further studies are needed to assess experimentally the relative importance of abiotic versus biotic factors in determining the patterns of smooth and winter flounder spatial distributions.

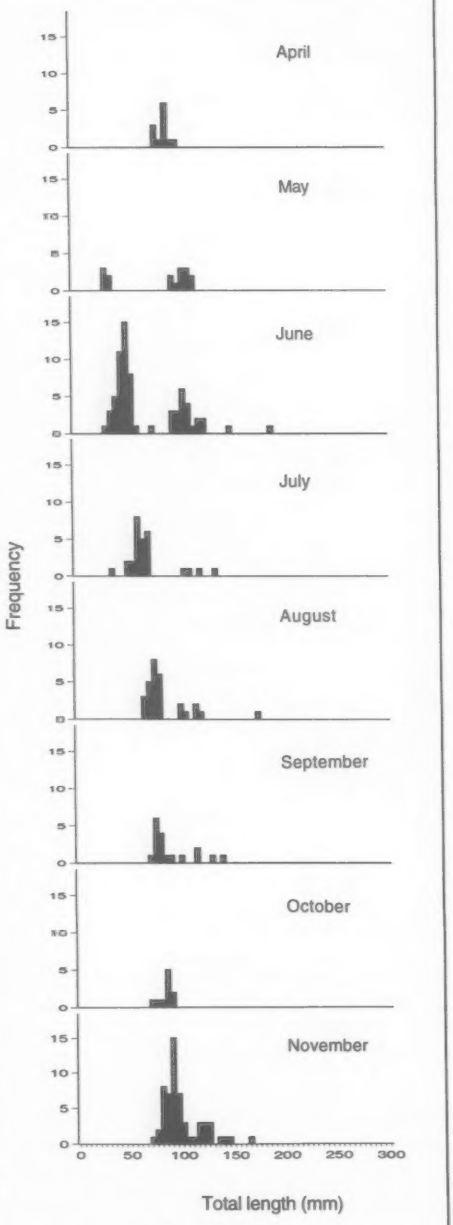

Figure 12

Monthly length frequencies (5-mm size classes) of smooth flounder, *Pleuronectes putnami*, at site 5, pooled over 1989–91.

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Energetics of larval red drum, *Sciaenops ocellatus*. Part II: Growth and biochemical indicators

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Abstract.—The effects of ration level and temperature on growth were determined for larval red drum, *Sciaenops ocellatus*, during its first two weeks of life. Larvae were raised in the laboratory at 20°C at a ration level of 5.0 prey/mL, at 25°C at ration levels of 0, 0.1, 1.0, and 5.0 prey/mL, and in growout ponds at 25°C and 32°C and at ration levels of 4–6 prey/mL. Growth was measured as standard length, wet mass, and dry mass. Proximate (water, ash, protein, and lipid) and elemental (C, N) composition was determined at larval ages of 0, 2, 4, 6, 10, and 14 d to provide caloric values for the growing larvae and to examine the relative importance of protein and lipid during tissue deposition in the very early life history of these larvae. Biochemical indicators of growth, RNA-DNA ratio, and activity of the metabolic enzyme lactate dehydrogenase (LDH) were examined in larvae reared at all temperature and ration combinations. The effectiveness of the biochemical indicators as proxies for growth was assessed by comparing the directly measured growth rates with RNA:DNA levels and LDH activity. Larvae fed a ration of 1.0 prey/mL or less did not survive past the age of eight days. Growth rate increased with increasing temperature, reaching a maximum of 60% body mass/d in growout ponds at 32°C. Protein level (percent ash free dry mass: %AFDM) increased with increasing age in all treatments where individuals exhibited positive growth, whereas lipid (%AFDM) showed a concomitant decline. Nitrogen (%AFDM) and carbon (%AFDM) varied directly with protein and lipid contents, respectively. Biochemical indicators of growth showed a significant correlation with growth rate. However, the character of the correlation changed with temperature. RNA-DNA ratios and enzymic activities were lower at higher temperatures for equivalent growth rates. Introduction of a temperature term into multiple regression equations improved the relation between growth and the biochemical proxies. LDH activity scaled with the size of larvae, whereas RNA:DNA showed no significant relation with size.

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Red drum, *Sciaenops ocellatus*, is an important species in commercial and recreational fisheries in the southeastern United States, particularly in the Gulf of Mexico. Declines in red drum stocks (Swingle, 1990) have stimulated considerable interest in the early life history of this species, resulting in stock enhancement programs and larval monitoring programs designed both to improve and continually to assess the status of the fish in the field. Studies on red drum and other species indicate clearly that growth during the pretransformation period of development is particularly critical to survival (Buckley, 1980; Holt et al., 1981, a and b; Holt and Arnold, 1983; Holt, 1990). The increase in size and mobility that characterizes development during the early larval period results in an increase in the size range of prey items available to the larvae as forage and a decrease in the size range of potential predators on the larvae.

Two variables with great potential to influence rates of growth are temperature and ration levels. As a subtropical species, red drum develop at temperatures greater than 20°C, grow rapidly, and have a greater energy demand for metabolic processes than do larvae developing in colder systems. For example, red drum eggs at 25°C hatch in 24 h, and larvae begin feeding in 48–72 h, whereas cold water species, such as Atlantic cod, *Gadus morhua*, and winter flounder, *Pleuronectes americanus*, developing at 4–8°C, spend 30 d as developing eggs.[1] High temperatures during early development stimulate rapid growth in red drum but leave them potentially more vulnerable to rapid starvation in absence of sufficient food. The interaction between temperature, ration level, and growth in size and calories, is an important part of the energetics of larval red drum, basic information which is unavailable for red drum and limited for other subtropical teleosts (Houde and Schekter, 1983).

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Objectives of the present study were four-fold. The first was to examine growth in size and energy in laboratory-reared red drum larvae, from egg to onset of transformation, at a single ration level (5 prey/mL) and at two temperatures (20 and 25°C). This examination was achieved by using direct measurements of standard length and mass with age; the biochemical composition and caloric value of the growing larvae were described by analyzing their proximate and elemental composition (water, ash, protein, lipid, carbon, and nitrogen). The second was to examine the relation of growth in size and energy as a function of ration level (0, 0.1, 1.0, and 5.0 prey/mL) at a single temperature (25°C). The third was to compare growth in size and energy in laboratory-reared larvae at 20 and 25°C and in the more heterogeneous conditions encountered by pond-reared larvae at 25 and 32°C. The fourth objective was to describe the relation between growth, temperature, and biochemical indicators of growth and condition: RNA:DNA ratios and activity of the key intermediate metabolic enzyme lactate dehydrogenase (LDH).

Methods and materials

Laboratory maintenance

Fertilized eggs were obtained from the Florida Department of Environmental Protection (FDEP) hatchery, Port Manatee, Florida. Broodstock were maintained at 25°C and 30 ppt. Eggs were obtained from five females and from separate spawnings, from November 1990 to November 1991, for all growth experiments described below. Broodstock females were similar in size, kept in highly controlled conditions, and fed well. Spawning was induced naturally by manipulation of photoperiod. As a consequence, eggs were very uniform in size, 0.9 to 1.0 mm in diameter.

Eggs were transported to the USF Marine Science Laboratory in St. Petersburg and sorted into 26-L experimental aquaria at a concentration of 2,500–3,000 individuals per aquarium. High mortality associated with first feeding resulted in a 30–40% reduction in initial numbers by day 3. Aquaria were placed in a photoperiod- and temperature-controlled incubator and maintained at either 20°C or 25°C and at a salinity of 30 ppt. Eggs were introduced to the 20°C temperature by slow exchange of water over a 60-minute period. A 13-h light and 11-h dark photoperiod was used throughout all experiments. Larvae were fed rotifers (*Brachionus plicatilis*) beginning at day 3 posthatch until flexion (approximately day 14), when experiments were terminated. Aquaria were aerated and a portion of the saltwater in each was changed daily.

Rotifers were obtained from Florida Aqua Farms, Dade City, Florida, and cultured according to the procedure of Hoff and Snell (1987). Rotifers were fed *Chlorella* once a day to avoid any loss in nutritional value. Seawater for culturing was obtained offshore in the Gulf of Mexico. The seawater was coarse-filtered, then treated with bleach (sodium hypochorite, 5.25%) to remove any additional plankton, and neutralized with sodium thiosulphate. Seawater salinity was adjusted with distilled water and Tropic Marine Seasalt to achieve a final salinity of 30 ppt.

Pond maintenance

Pond-reared red drum larvae were obtained from the FDEP growout ponds, Port Manatee, Florida. Larvae from a single spawn were added to the plankton-rich ponds within 24 hours after hatching and allowed to grow. Two ponds, one at 25°C and another at 32°C, were sampled for the first 18 days of life of the red drum larvae. Temperature was monitored twice daily; the average temperature for the two-week sampling period was used to characterize the ponds.

Prey items in the ponds were monitored by sieving water samples into two size categories: 35–220 µm (copepod nauplii, rotifers, and small copepods) and larger than 220 µm (copepods); prey were then counted in 200-mL aliquots of each size range. The concentration of prey between 35 and 220 µm was 3–5 prey/mL, whereas that greater than 220 µm was 0.5–1 prey/mL in both the 25°C and 32°C ponds.

Growth versus prey density

Eggs from a single spawn were divided into four 26-L aquaria for experiments on growth versus prey density at 25°C. Prey were provided at four densities, 0, 0.1, 1.0, and 5.0 prey items per mL, from first feeding (day 3) through the start of flexion (day 14). Prey concentrations were monitored twice daily by removing a 25-mL sample from each aquarium, counting the number of prey in 5-mL aliquots, and taking the average. Prey concentrations were adjusted as necessary. Larvae reared at 20°C were fed prey at a ration level of 5.0 prey items per mL.

Standard length measurements Growth in standard length was monitored according to prey concentration. Aquaria with 0, 0.1, and 1.0 prey/mL were sampled daily. Aquaria with 5.0 prey/mL were sampled every other day, and ponds were sampled every third day. The samples were taken each morning before the larvae began to feed. Standard length

of five individual larvae that had been anesthetized with MS-222 was measured with the aid of a dissecting microscope. Standard length was considered to be the distance from the snout to the tip of the tail in preflexion larvae and from the snout to the tip of the notochord in post-flexion larvae.

Mass measurements Growth in mass was monitored at the same intervals as those used for standard length. At each monitoring interval, 30 individuals were removed for wet, dry, and ash-free dry mass analysis. To determine mass, larvae were first separated into three groups of ten. Each group of larvae was filtered onto a preweighed 0.5-cm Whatman glass fiber filter (made with an office hole-punch) that was placed in a custom-made miniaturized vacuum funnel. Larvae were then rinsed very briefly by introducing distilled water into the funnel with a pasteur pipette and by removing the water immediately with the vacuum filter. To minimize evaporation, samples were immediately placed in preweighed microcentrifuge tubes which were then weighed to the nearest µg on a Mettler electrobalance to determine wet mass. Specimens were dried at 60°C to a constant mass (about 24 h) to determine dry mass.

Average proximate and elemental composition of prey items

Rotifers were collected in bulk from two 28-L culture bags (approximately 50 mg dry mass/bag) for determination of proximate and elemental composition. Proximate composition (water, ash, protein, and lipid content) was determined by using the methods of Stickney and Torres (1989) and Donnelly et al. (1990). Elemental composition was determined by using a C:H:N analyzer.

Average proximate and elemental composition of fish larvae

Methods used to estimate the proximate and elemental composition of fish larvae were the same as those used for prey. Larvae were obtained in bulk (50 mg dry mass) for each day sampled. Each pond was sampled from the hatchery at 0, 2, 6, 10, and 14 days. Laboratory-raised larvae were sampled at prey concentrations of 0, 0.1, 1.0, and 5.0 prey/mL at 0, 2, 6, 10, and 14 days for each of four spawns. Protein and lipid values as percent ash-free dry mass (%AFDM) were multiplied by individual ash-free dry mass to obtain concentrations as mg/individual. The instantaneous protein growth rate (Gpi) was calculated by using the formula from Buckley (1982):

$$Gpi = \frac{\ln M_{t2} - \ln M_{t1}}{t_2 - t_1} \times 100,$$

where M = mass in mg; and
t = age in d.

Caloric content of prey and larvae

Caloric content was calculated from proximate compositional data of the rotifers and larvae by using a value of 0.0048 cal/µg for protein and 0.0095 cal/µg for lipid (Brett and Groves, 1979).

RNA-DNA ratio

Ten to 20 individuals were removed for analysis of RNA:DNA content each time sampling occurred for measurements of mass. Larvae were filtered onto preweighed Whatman glass-fiber filters, rinsed with distilled water, weighed, placed in microcentrifuge tubes, and frozen at –80°C until analysis. RNA:DNA was analyzed by first homogenizing the freshly thawed groups of larvae in 1.2 M NaCl, then by using the sequential enzymatic method of Bentle et al. (1981) to determine RNA:DNA.

Activity of lactate dehydrogenase

Larvae were sampled in bulk (minimum 10–20 mg wet tissue mass) every day at a prey concentration of 0 prey/mL. Samples were taken at 0, 2, 6, 10, and 14 days for larvae fed 5 prey/mL and for those collected in the growout ponds. Tissue was introduced frozen into the homogenizing medium, ice-cold Tris/HCL buffer (10 mM, pH 7.5 at 10°C), and homogenized by hand at 0 to 4°C with conical glass homogenizers having ground-glass contact surfaces (Kontes Glass Co., "Duall" models). Homogenates were centrifuged at 4,500 ×g for 10 minutes and the supernatants saved for enzyme analysis.

L-Lactate dehydrogenase (LDH, EC 1.1.1.27; Lactate: NAD+ Oxidoreductase) activity was assayed in the pyruvate reductase direction by using methods described in Torres and Somero (1988) at a temperature of 25°C. Enzyme activity was expressed as units/gWM (wet mass), where a unit was 1 µmole of substrate converted to product per minute.

Statistical analyses

Simple regressions for each relationship were fitted by using the least-squares method (Statgraphics Plus, Manugistics Corporation). Data from treatment groups were compared by using one-way analysis of variance (ANOVA). Differences between the means

were determined by using the least-significant-differences multiple range test. Multiple regression analysis (Statgraphics Plus, Manugistics Corporation) was used to examine the relation between the observed protein growth rates and the following combinations of factors: the three experimental temperatures (20°C laboratory, 25°C laboratory, 25°C pond, 32°C pond), the three ration levels at 25°C (0, 5 prey/mL, and pond), RNA:DNA, and LDH activity.

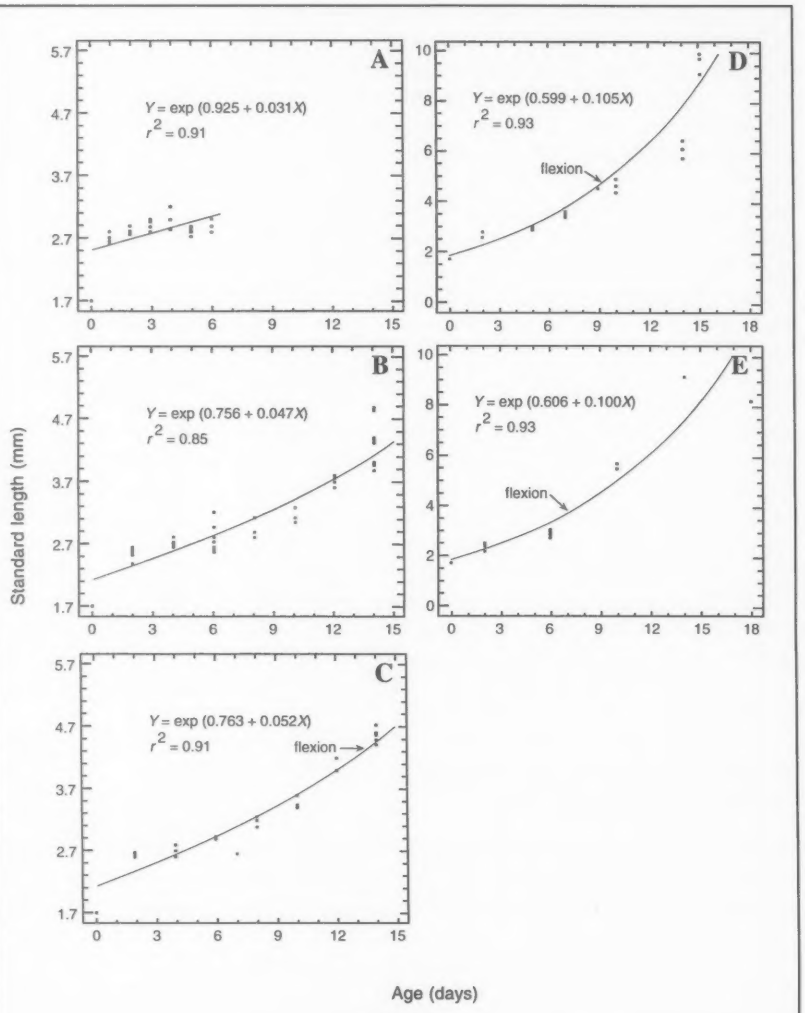

Figure 1

Growth in standard length of red drum larvae raised in the laboratory and in growout ponds: (**A**) laboratory, 25°C and 0 prey/mL; (**B**) laboratory, 20°C and 5 prey/mL; (**C**) laboratory, 25°C and 5 prey/mL; (**D**) pond, 25°C; and (**E**) pond, 32°C.

Results

Growth rate versus prey density

Standard length Starved red drum larvae (0 prey/mL) kept at 25°C increased in standard length even as they were declining in dry mass (Figs. 1 and 2; Table 1). The average size at death on day 6 was 2.89 mm, which corresponds to a daily increase of 0.075 mm/day for days 2–6. Surprisingly, these values were similar to those for larvae fed at 5.0 prey/mL at 25°C, which attained an average length of 2.90 mm by day 6.

Growth in larvae fed ad libitum increased with increasing temperature in both the laboratory and ponds. At 20°C, day-14 larvae averaged 4.13 mm, and very few of the larvae exhibited flexion of the notochord (Fig 1; Table 1). Laboratory-reared individuals at 25°C grew to an average of 4.45 mm by day 14. Mean masses at day 14 were significantly different between the two temperatures (ANOVA: df=1, F=60.3, P<0.001). Flexion of the notochord within the tail region had begun by day 14 at 25°C, indicating the onset of transformation. Larvae raised in ponds at 25°C averaged 6.45 mm at day 14. Notochord flexion in these larvae began on day 9 or 10, when the larvae were at a size between 4.11 and 4.50 mm. Pond-reared larvae at 32°C had reached an average standard length of 7.48 mm at day 14. Notochord flexion occurred on day 7 or 8, when larvae were at a length of 4.53 mm (similar to the size at flexion of the laboratory-raised larvae) and at an earlier chronological age than that of the laboratory-raised individuals.

Mass measurements Growth in mass of laboratory-reared larvae at ration levels less than 5.0 prey/mL (0, 0.1, and 1.0 prey/mL) at 25°C was negative, and no larvae survived more than 8 days (Fig. 2). Slopes

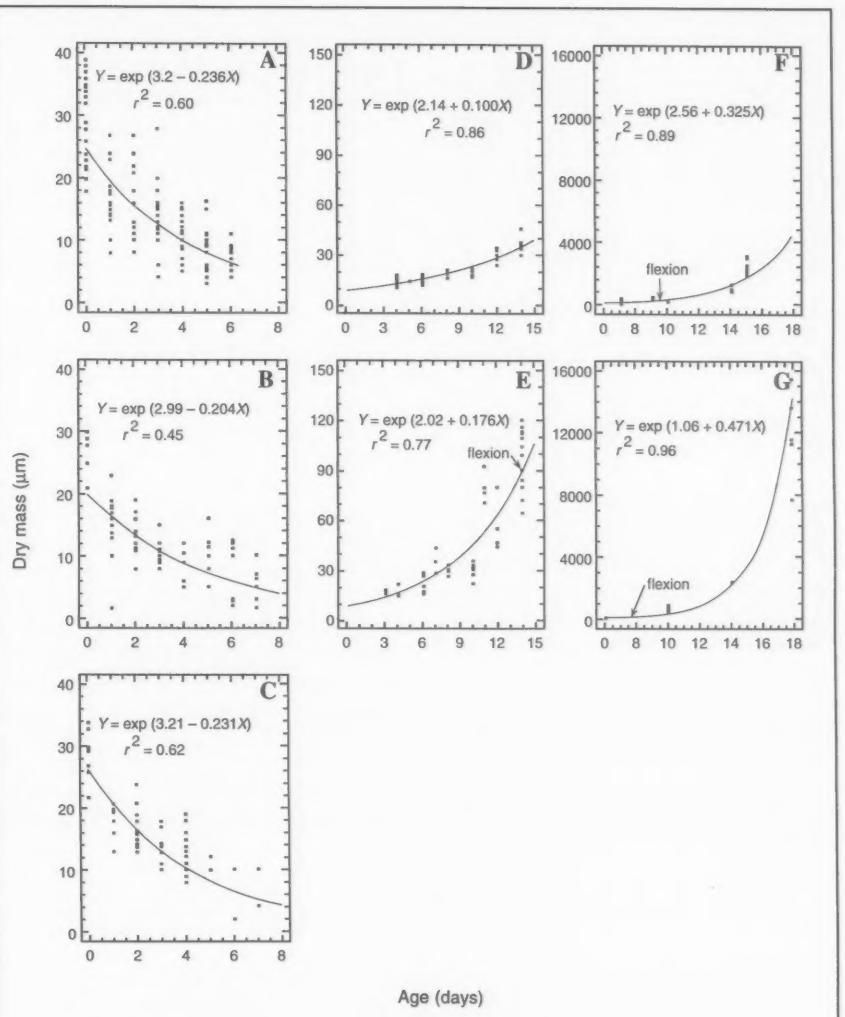

Figure 2

Growth in dry mass of red drum larvae raised in the laboratory and in growout ponds: (**A**) laboratory, 25°C and 0 prey/mL; (**B**) laboratory, 25°C and 0.1 prey/mL; (**C**) laboratory, 25°C and 1 prey/mL; (**D**) laboratory, 20°C and 5 prey/mL; (**E**) laboratory, 25°C and 5 prey/mL; (**F**) pond, 25°C; and (**G**) pond, 32°C.

of the three curves describing the time-dependent decline in individual dry biomass at the three ration levels were not significantly different (Student's *t*-test: $P>0.05$). Growth in dry mass at a ration level of 5.0 prey/mL was significantly higher than at the three lower ration levels (0, 0.1, and 1.0 prey/mL).

In contrast, wet masses in 2–6 day-old larvae were slightly lower at ration levels of 0, 0.1, and 1.0 prey/mL than at 5.0 prey/mL but were not significantly different. This finding indicated that body water content was increasing in larvae maintained at the three lower ration levels.

Larvae held at 25°C and fed 5.0 prey/mL had higher growth rates than those raised at 20°C at the same ration level (Fig. 2; Table 1). Growth averaged 2.86 µg/day for the first two weeks of life in larvae raised at 20°C and 10.09 µg/day for larvae reared at 25°C. Expressed as a percent increase in mass, larvae reared at 20°C increased 10.5 %BM/d and those reared at 25°C increased 19.3 %BM/d.

Table 1

Proximate and elemental composition and energetic density of red drum larvae determined at different levels of ration, temperature, and age. (nd = no data; DM=dry mass; WM = wet mass; AFDM = ash-free dry mass). Parenthetical values (SD, *n*) represent standard deviations and sample sizes below each of the mean values in the table.

Ration (prey/mL)	Temp C°	Day	Standard length (mm)	Wet mass (µg)	Dry mass (µg)	Calories per ind.	% Water	AFDM as %DM	Protein		Lipid		Carbon	Nitrogen	Calories/ mg DM
									% WM	% AFDM	% WM	% AFDM	% AFDM	% AFDM	
0	25	0	1.70 (0.05, 5)	447.00 (86.10, 18)	29.28 (7.08, 18)	0.1063	94.4 (1.4, 15)	91.3 (4.5, 15)	2.58 (0.80, 15)	43.84 (4.47, 15)	1.22 (0.50, 15)	19.72 (4.16, 15)	51.83 (3.98, 3)	10.31 (0.55, 3)	3.631
0	25	1	2.69 (0.09, 5)	274.80 (176.00, 15)	16.36 (4.52, 15)	0.0698	92.8 (1.2, 5)	94.5 (0.2, 5)	4.01 (0.81, 5)	57.33 (5.53, 5)	1.23 (0.01, 5)	18.45 (2.08, 5)	48.93 (0.93, 3)	10.41 (0.52, 3)	4.266
0	25	2	2.83 (0.06, 5)	140.40 (24.30, 15)	16.66 (5.54, 15)	0.0725	90.8 (0.7, 10)	90.7 (1.8, 10)	4.71 (0.56, 10)	55.68 (3.79, 10)	1.74 (0.47, 10)	22.39 (6.02, 10)	53.99 (1.10, 4)	9.70 (0.48, 4)	4.353
0	25	3	2.91 (0.09, 5)	126.20 (26.30, 15)	13.48 (4.93, 15)	0.0596	92.8 (1.0, 10)	90.6 (0.9, 10)	3.90 (0.47, 10)	59.68 (5.41, 10)	1.34 (0.29, 10)	21.24 (6.06, 10)	49.79 (4.84, 3)	9.80 (0.50, 3)	4.423
0	25	4	3.01 (0.15, 5)	120.60 (21.70, 15)	10.99 (3.31, 15)	0.0438	91.1 (0.8, 10)	90.1 (1.2, 10)	4.69 (0.43, 10)	58.74 (2.48, 10)	1.32 (0.26, 10)	16.86 (4.35, 10)	49.79 (1.92, 3)	10.05 (0.13, 3)	3.983
0	25	5	2.81 (0.07, 5)	103.30 (26.00, 15)	8.13 (3.86, 15)	0.0308	91.8 (0.6, 5)	89.6 (0.7, 5)	4.33 (0.36, 5)	62.89 (1.20, 5)	0.83 (0.17, 5)	12.67 (1.72, 5)	46.06 (5.81, 3)	10.60 (0.23, 3)	3.783
0	25	6	2.89 (0.08, 5)	91.50 (20.90, 15)	7.00 (1.80, 15)	0.0278	91.0 (1.0, 5)	93.1 (1.6, 5)	5.21 (0.26, 5)	62.47 (3.28, 5)	1.14 (0.01, 5)	13.29 (1.28, 5)	nd	nd	3.967
5	20	0	1.70 (0.05, 5)	363.30 (109.90, 0)	29.40 (4.26, 0)	0.1230	95.4 (0.5, 5)	94.8 (7.4, 5)	2.35 (0.44, 5)	43.74 (4.27, 5)	0.89 (0.18, 5)	24.34 (1.28, 5)	nd	nd	4.182
5	20	2	2.54 (0.10, 5)	156.80 (22.30, 10)	15.52 (3.78, 10)	0.0651	90.3 (1.1, 5)	93.2 (2.3, 5)	4.20 (0.49, 5)	53.08 (4.86, 5)	1.70 (0.31, 5)	20.54 (3.12, 5)	nd	nd	4.194
5	20	6	2.79 (0.21, 5)	192.00 (106.50, 10)	15.64 (1.87, 10)	0.0509	90.3	91.0	4.71	53.19	0.95	10.78	nd	nd	3.255
5	20	10	3.14 (0.10, 5)	233.80 (20.30, 10)	20.43 (2.15, 10)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
5	20	14	4.33 (0.12, 5)	384.40 (47.30, 10)	35.91 (4.57, 10)	0.1056	85.7	87.7	6.50	51.73	1.15	9.17	nd	nd	2.943
5	25	0	1.70 (0.09, 5)	385.80 (17.40, 5)	35.25 (1.26, 3)	0.1373	95.7 (0.7, 5)	97.4 (2.4, 5)	1.81 (0.01, 5)	43.39 (0.67, 5)	0.84 (0.01, 5)	20.18 (2.16, 5)	46.66	10.46	3.896
5	25	2	2.64 (0.03, 5)	206.30 (85.20, 5)	18.67 (0.58, 5)	0.0849	93.5 (2.1, 5)	91.8 (1.3, 5)	3.27 (0.01, 5)	54.64 (1.24, 5)	1.49 (0.01, 5)	24.54 (0.02, 5)	49.88	10.29	4.548
5	25	6	2.90 (0.02, 5)	207.80 (74.50, 5)	20.33 (5.47, 5)	0.0775	88.0	91.3	5.79	52.91	1.89	17.23	45.77 (0.41, 3)	12.03 (0.14, 3)	3.813
5	25	10	3.46 (0.10, 5)	273.50 (121.00, 6)	29.71 (4.23, 6)	0.1005	85.8	89.4	7.07	55.59	1.49	11.74	42.92	11.91	3.382
5	25	14	4.53 (0.12, 5)	682.80 (181.40, 15)	98.92 (18.43, 15)	0.3120	87.8 (0.1, 3)	86.7 (0.7, 8)	5.59 (0.08, 8)	55.13	1.22 (0.16, 8)	11.64	45.73 (1.17, 3)	12.27 (0.50, 3)	3.253
Pond	25	0	1.72 (0.04, 5)	352.30 (71.00, 5)	28.91 (4.43, 5)	0.1008	95.0	92.56	1.74	42.70	0.72	18.08	nd	nd	3.450
Pond	25	2	2.70 (0.12, 5)	179.00 (62.60, 5)	15.67 (2.71, 5)	0.0738	91.4	93.1	4.42	56.21	1.72	24.82	nd	nd	4.707
Pond	25	7	2.93 (0.06, 5)	334.05 (34.80, 5)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pond	25	10	4.70 (0.03, 5)	2833.80 (381.50, 5)	137.30 (23.20, 5)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pond	25	14	6.30 (0.31, 5)	6010.00 (542.50, 5)	983.30 (167.60, 3)	3.1318	91.7	87.6	10.18	56.22	1.99	9.87	nd	nd	3.185
Pond	32	0	1.75 (0.04, 5)	nd	nd	nd	93.9	97.2	nd	45.62	nd	24.98	47.70	9.10	nd
Pond	32	2	2.37 (0.14, 5)	187.80 (72.40, 5)	17.32 (4.78, 5)	0.0806	92.7	91.3	3.60	54.00	1.76	26.36	51.53	10.51	4.652
Pond	32	6	2.89 (0.12, 5)	374.30 (83.50, 5)	136.20 (35.30, 5)	0.5476	88.5	91.6	6.92	65.61	1.38	13.05	nd	nd	4.021
Pond	32	10	5.65 (0.10, 5)	5491.00 (1802.00, 5)	583.00 (168.40, 5)	2.1330	88.0	87.1	6.71	64.16	1.23	11.70	44.35	11.86	3.658
Pond	32	14	7.48 (0.27, 3)	nd	2127.80 (293.00, 3)	6.9808	89.3	81.7	5.00	64.16	nd	9.87	46.79	13.14	3.282

Growth rates for pond-raised red drum larvae (Fig. 2) were far greater than those for larvae fed rotifers in the laboratory, owing almost certainly to the greater prey diversity in the ponds. Larvae raised at 25°C in the ponds increased in size an average of 81.9 µg/day over 14 days: a 47.2 %BM/d increase. Larvae raised at 32°C in the ponds increased an average of 799.22 mg/day: a 60.2 %BM/d increase; an increase of 10°C in the pond environment resulted in a two- to three-fold increase in absolute daily mass gain.

Proximate and elemental composition of prey

Brachionus plicatilis raised on *Chlorella* exhibited a protein level of 32.71% and a lipid level of 9.37% of its ash-free dry mass (AFDM). Carbohydrate level was low, averaging 2.84% (AFDM). The unrecovered mass was assumed to be due to refractory structural molecules that were not assayed for, e.g. chitin. Using a figure of 0.24 µg for average individual biomass (Hoff and Snell, 1987) and the caloric values for protein, lipid, and carbohydrate of Brett and Groves (1979, see methods section), we suggest that each rotifer has an average energetic value of 0.000526 calories.

Elemental composition of the rotifers showed that the percent carbon was 42.02 %AFDM and the percent nitrogen was 10.41 %AFDM. The carbon-nitrogen ratio was 3.56:1.

Proximate and elemental composition of larvae

Proximate composition can be expressed in three ways: as a percent of wet mass (%WM), a percent of ash-free dry mass (%AFDM), and as the total content per larva (mg/individual). Table 1 shows the changes in proximate composition (%WM and %AFDM) as a function of ration level and age of the larvae; total content (mg/individual) is reported below in the text.

Red drum eggs exhibited large water content (94.65 %WM), a high protein content (42.17 %AFDM) and an intermediate lipid content (19.35 %AFDM)(Table 1). Carbohydrate, generally an extremely small fraction of the overall proximate composition of marine species, proved to be so in this case as well (0.47 %AFDM).

Viewed as a fraction of the total body mass of each larva, the protein level (%AFDM) shows an increase through time at zero ration (43.84% to 62.47%) accompanied by a reduction in lipid (19.72% to 13.29%), which indicates that, in starving larvae, lipid was used in preference to protein for energy production. On a µg/individual basis, protein actually decreased in starved larvae from 8.44 µg/individual in newly hatched larvae to 4.07 µg/individual for 6-day-old starved larvae (Table 2). Lipid values declined from 2.59 µg/individual on day 3 to 0.87 µg/individual on day 6. Percent water remained high until death at day 6, averaging 91.0% throughout the survival period.

Table 2

Protein content, protein growth, RNA:DNA, and LDH in red drum larvae. Protein content was calculated from values reported in Table 1. Where values for % protein or % ash-free dry mass were missing, nearest neighbor values were used. Protein growth was calculated as described in text. RNA:DNA values are the mean for all RNA:DNA measurements within the last 2 d of the age interval shown in the table. They are reported as mean ±SD (n). Values for the 25°C pond were taken at day 7 instead of day 6 and calculated accordingly. All (6–14) day RNA:DNA values are significantly different ($P<0.05$, Students t). nd = no data

	Day															
	2	6	10	14	2–6	6–10	10–14	2–14	6–14	6	10	14	6–14	6	10	14
	Protein (ug/indiv)				Protein growth (%/d)					RNA:DNA				LDH (units/gWM)		
Starved	8.44	4.07	nd	nd	–18.20	nd	nd	nd	nd	0.68±0.35 (14)	nd	nd	nd	7.48	nd	nd
5/mL at 2°C	7.66	7.55	9.29	16.34	–0.40	5.20	14.10	63.00	9.50	1.51±0.43 (6)	1.45±0.24 (4)	1.49±0.14 (4)	1.50±0.30 (14)	9.19	14.44	19.69
5/mL at 25°C	9.36	9.77	14.80	47.33	1.10	10.40	29.10	13.50	19.70	1.15±0.20 (2)	1.19±0.32 (3)	1.26±0.19 (7)	1.25±0.21 (12)	15.06	24.33	33.60
Pond 25°C	8.16	15.00	67.70	484.30	12.10	50.20	49.20	34.00	49.60	2.89±0.51 (4)	3.06±0.81 (4)	3.56±0.27 (2)	3.09±0.62 (10)	18.94	30.86	42.78
Pond 32°C	8.51	82.20	324.60	1,116.20	56.70	34.30	30.90	46.00	32.60	1.19	2.21	1.39	1.60±0.54	12.69	19.44	26.19

The counterpoint to 0-ration data is provided by the data at 5.0 prey/mL at 20°C and 25°C (Table 1). The data clearly demonstrate accumulation of energy as protein and little storage of lipids. Larvae raised at 20°C increased in protein level (%AFDM) from 43.74% as eggs to 51.73% as day-14 larvae. Lipid levels decreased (%AFDM) from 24.34% in eggs to 9.17% in day-14 larvae. Protein concentrations increased from 7.66 µg/individual at day 2 to 16.34 µg/individual at day 14. Lipid concentrations increased from 1.53 µg/individual at day 6 to 2.89 µg/individual at day 14. An identical pattern was observed in larvae reared at 25°C (Table 1).

The data set for proximate composition values collected on pond-raised larvae was smaller than ideal owing to problems in obtaining adequate sample sizes from the ponds. However, the data on accumulated protein and lipid concentrations give an excellent indication of maximum growth. Pond-raised larvae at 25°C and 32°C showed faster accumulation of total protein and lipid than larvae raised in the laboratory (Table 1). Protein levels of larvae increased in %AFDM from 42.70% and 45.62%, in eggs, to 56.22% and 64.16% in day-14 larvae, at 25°C and 32°C, respectively. Lipid levels (%AFDM) decreased from 24.98% to 9.87% at 32°C; decreases in lipid percentages were also observed in the 25°C pond and in the laboratory-raised larvae.

Pond-reared larvae at 25°C increased in total protein content from 8.16 µg/individual at day 2 to 484.30 µg/individual at day 14, whereas those kept at 32°C increased from 82.20 µg/individual at day 7 to 1,116.20 µg at day 14 (Table 2). Thus, an increase in 10°C resulted in a three-fold increase in protein (µg/individual) in 2-week-old larvae raised in the ponds. Lipid values for larvae raised at 25°C and 32°C were also much higher than those for larvae raised in the laboratory; day-14 pond larvae on average had lipid contents of 85.06 µg/individual and 171.44 µg/individual, respectively.

Carbon (%AFDM) remained about the same with age in all rearing conditions (Table 1), whereas nitrogen (%AFDM) remained fairly constant or increased with age at all ration levels. Carbon-nitrogen (C:N) ratios were higher in larvae kept at a ration level of 0 prey/mL than in larvae raised either at 5.0 prey/mL or in the ponds, indicating that protein commanded the largest fraction of the starved larva's mass. Values for C:N remained high in starved larvae until death at day 5 (4.35 ± 0.46). Pond-raised larvae had values similar to those observed in larvae reared on 5.0 prey/mL in the laboratory but were slightly lower at day 14 (3.56 vs. 3.73). Caloric contents of the larvae were calculated from protein and lipid content by using the conversion factors in Brett and Groves (1979) and are reported in Table 1.

Protein-specific growth and biochemical indicators

Table 2 summarizes results for growth in protein (absolute and instantaneous) and the two biochemical indicators, RNA:DNA and LDH activity, for easy comparison. Instantaneous growth shows an interesting trend with the age interval chosen for calculation. If growth in protein was calculated from day 2 to day 14, larvae exhibit the trends discussed previously for growth in dry mass (see results) where lowest growth was observed in the 20°C laboratory treatment and highest in the 32°C ponds. If instantaneous growth was calculated instead for the interval from day 6 to day 14, the highest growth was observed in the 25°C ponds (Table 2) and would suggest that the growth spurt during the first 4 d of feeding in the 32°C pond was important in determining growth during the larvae's first 14 d of life.

RNA-DNA ratio RNA:DNA in each treatment showed a decline from a high value typical of the yolk-sac stage (day 1: grand mean for all treatments 4.27 ± 0.83; $\bar{x} \pm$ SD) to a plateau at day 4 that characterized the treatment and showed no significant change over the remaining 10 days (Fig 3; Table 2). In starved larvae RNA:DNA reached a plateau at a value of 0.7, indicating that protein synthetic capacity was severely diminished after that time. RNA:DNA in larvae raised at 5.0 prey/mL in the laboratory showed a gradual decline to a plateau of 1.5 at 20°C and 1.3 at 25°C (Fig 3; Table 2); values at the plateau were significantly different between the two temperatures (ANOVA: df=25, F=6.31, P=0.019)

Pond-raised larvae had higher growth rates than laboratory-reared individuals (Figs. 1 and 2; Tables 1 and 2), and values for RNA:DNA were much greater in the 25°C pond than in any of the laboratory treatments (Fig. 3). Larvae raised in the ponds at 25°C had a value of 3.6 at 2 weeks of age, whereas those reared at 32°C averaged 1.5 at day 14. RNA:DNA values were significantly different between the two pond treatments (ANOVA: df=12, F=14.19, P=0.003).

Three treatments took place at a temperature of 25° C: starved, 5 prey/mL, and pond. If protein growth rates and RNA:DNA are compared between laboratory and ponds at 25°C (Table 2), there is an excellent correlation between protein growth and RNA:DNA. Instantaneous protein growth rate in the laboratory was 13.5%/d from day 2 to day 14; in the pond it was 34%/d over the same interval: an increase of 2.5 fold. RNA:DNA showed an increase of 1.3 to 3.0 in laboratory- versus pond-reared larvae over the same interval with a similar 2.3-fold increase. The negative growth observed in starved larvae at 25°C

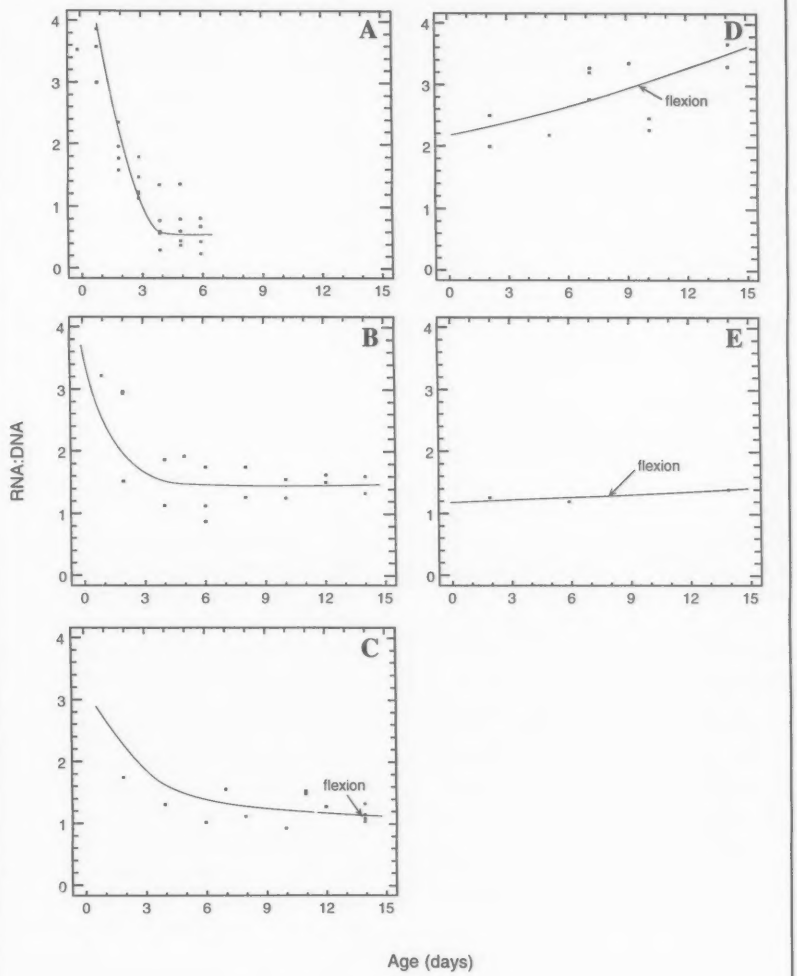

Figure 3

RNA:DNA of red drum larvae raised in the laboratory and in growout ponds: (**A**) laboratory, 25°C and 0 prey/mL; (**B**) laboratory, 20°C and 5 prey/mL; (**C**) laboratory, 25°C and 5 prey/mL; (**D**) pond, 25°C; and (**E**) pond, 32°C.

(–18%/d) also showed a much lower value for RNA-DNA ratio : 0.7:1. Differences in RNA:DNA between the three treatments were highly significant (ANOVA: df=35, F=107.6, P=0.000).

Overall, RNA:DNA was only a modest predictor of protein growth. Regression analysis of protein growth (y, % per d) versus RNA:DNA (x), by using all the values in Table 2, showed a marginal fit (y = –3.64 +14.76x; P=0.01; r^2=0.34). However, as discussed above, within a temperature, RNA:DNA was an excellent predictor of instantaneous protein growth, and this was borne out in a regression using only the data collected at 25°C: y (% per d) = –12.43 + 17.39 (RNA:DNA); P=0.01; r^2=0.64. The performance of RNA:DNA as an overall predictor was improved significantly by using a multiple regression equation with a temperature term (Table 3).

LDH Activity LDH activities of laboratory-raised larvae increased with age at ration levels of 0 and 5.0 prey/mL and with temperatures of 20°C and 25°C (Fig 4). Larvae that were starved continued to pro-

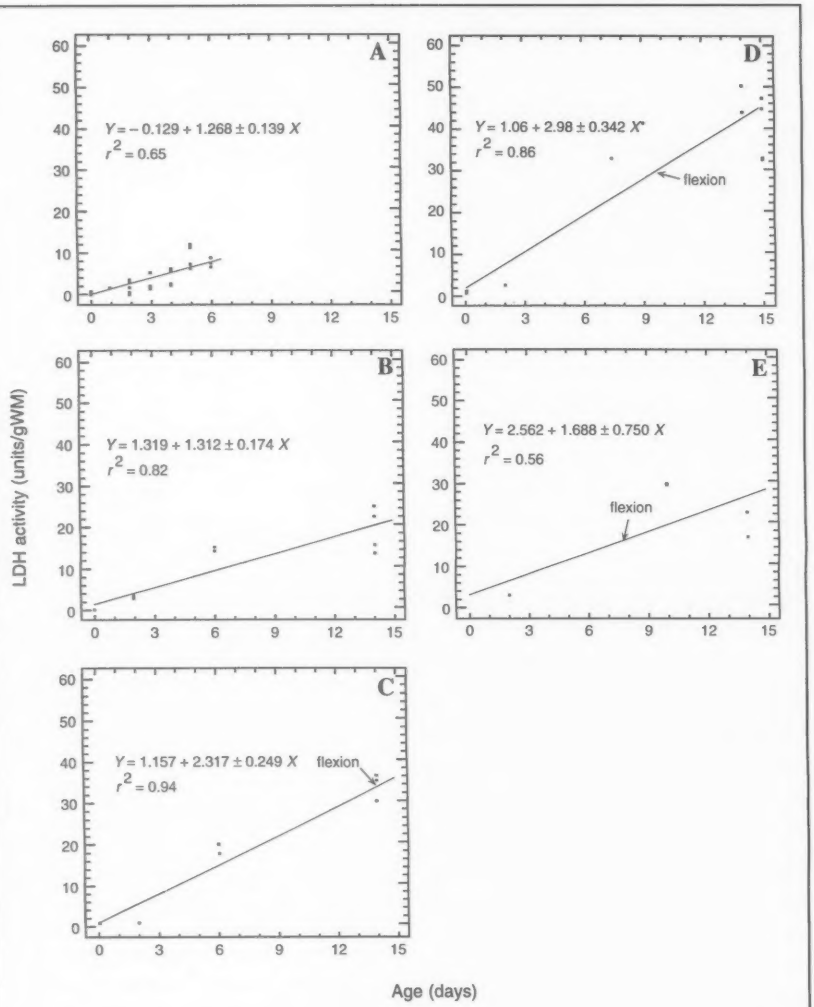

Figure 4

LDH activity (units/gWM) in red drum larvae raised in the laboratory and in growout ponds: (**A**) laboratory, 25°C and 0 prey/mL; (**B**) laboratory, 20°C and 5 prey/mL; (**C**) laboratory, 25°C and 5 prey/mL; (**D**) pond, 25°C; and (**E**) pond, 32°C.

duce LDH, although at lower concentrations than those for fed individuals, until death at day 6. Larvae reared at 20°C had LDH values of 20–25 units/gWM at day 14. These LDH activities were slightly lower than those for larvae raised at 25°C, which had LDH values of between 30 and 35 units/gWM at day 14. Larvae reared at 25°C in the ponds averaged LDH activities of 40–50 units/gWM, higher than the values for larvae raised at 32°C, which averaged 25–30 units/gWM, and higher than the values for larvae reared in the laboratory.

Like RNA:DNA, LDH activity taken overall was only a modest predictor of protein growth rate: y (% per d) = –8.17 + 1.39 (LDH); P=0.02; r^2=0.39. However, within a temperature, its performance as a predictor was much improved. A regression using only the 25°C data yielded an excellent coefficient of determination: y (% per d) = –28.74 + 1.94 (LDH); P=0.003; r^2=0.86. A multiple regression with a temperature term improved its use as an overall predictor (Table 3).

Table 3

Multiple regressions describing instantaneous protein-specific growth (Y; %/d) in red drum larvae versus temperature (T), ration (0/ml, 5/mL, and pond), RNA:DNA, and LDH activity (units/gWM). Only significant regressions are presented ($P < 0.05$).

Equation	X_i	X_{ii}	Y	n	r^2	P
1	T		$Y = 2.71X_i - 49.52$	20	0.21	0.023
2	Ration		$Y = 6.35X_i - 8.53$	20	0.45	0.001
3	T	Ration	$Y = 2.07X_i + 5.67X_{ii} - 58.34$	20	0.57	0.022
4	RNA:DNA		$Y = 14.76X_i - 3.64$	17	0.30	0.013
5	T	RNA:DNA	$Y = 2.59X_i + 14.61X_{ii} - 69.41$	17	0.56	0.007
6	LDH		$Y = 1.39X_i - 8.17$	13	0.34	0.022
7	T	LDH	$Y = 2.55X_i + 1.26X_{ii} - 70.35$	13	0.57	0.034

Discussion

Growth versus prey density

Standard length and mass measurements The basic pattern of growth and development in red drum larvae, e.g. in size at flexion, was similar for larvae under a wide variety of rearing conditions. Within the basic blueprint, growth and development of red drum larvae fed to satiation could be accelerated or retarded according to the rearing temperature.

Thus, larvae raised in the laboratory and the ponds underwent metamorphosis at roughly the same size, independent of the age of the larvae. In the case of the 32°C pond, day-7 larvae were already the size of day-14 larvae reared at 25°C in the laboratory, and were at the same stage of development. Similarly, dry mass at transformation was approximately the same in the laboratory and ponds, despite the differences in chronological age.

Proximate and elemental composition of larvae

Red drum larvae, whether fed to satiation or starved, depleted their lipid level from 40% to 50% by day 6. The increase in protein (%AFDM) reflected the decline in lipid and was most evident in the starved red drum larvae. Larvae that have been starved conserve protein as musculature until the time of death. Conservation of muscular proteins allows the animal to swim as long as possible before complete muscle atrophy, or "point of no return," allowing the larvae to search out prey in other, possibly more productive, areas.

The loss of dry mass in starving larvae, compared to fed larvae of equal age, reflected the catabolism of lipid and protein (Wallace, 1986). A similar, but less severe, drop in lipid was observed in all rearing conditions and has been observed in other species of fish. For example, Fraser et al. (1987) found that larval Atlantic herring had a lipid level of 23% dry mass (176 μg) one day after hatching decreasing to 11% (221 μg) by day 16. Those percentages were similar to those found for red drum larvae in the present study (20.18% to 11.74%) over the first two weeks of life. It is likely that lipid serves as a buffer fuel during the early life history of red drum. It is not accumulated. When high-quality food energy is available in excess, larval red drum larvae grow faster rather than accumulate an energy reserve. This is best exemplified by the differences in larvae growing at 25°C in the laboratory and 25°C in the ponds.

Elemental composition agreed well with other published values for red drum (Lee et al., 1988) and larval herring of similar size (Ehrlich, 1974, a and b; 1975) as well as with our own results on proximate composition (Table 1). Larvae that are growing normally, as in the 5.0 prey/mL experiments and the ponds, show greater increases in protein than in lipid. The increase in %N with age, and the declining %C, mirrored the changes (protein increase, lipid decrease) in proximate composition. This changing elemental composition resulted in a declining C:N in normally growing larvae. Starving individuals had slightly higher C:N than fed individuals as a result of their diminished protein synthesis. Larvae raised in the ponds have the lowest C:N as a result of the high protein levels relative to lipid. Thus, the C:N can be used as an indicator of physiological status in developing fish. It should be noted, however, that this ratio applies in the opposite fashion to adult fish. A declining C:N in older fish indicates starvation where lipid is laid down as an energy reserve and is combusted before protein. The rapidly accumulating musculature of a healthy, growing fish larva results in a declining C:N, giving the appearance of starvation when, instead, this ratio indicates that protein is accumulating at a faster rate than lipid.

Protein-specific growth and biochemical indicators

RNA-DNA ratio Our values for RNA:DNA fall at the low end of the range of ratios reported in the literature for larvae reared under a variety of different conditions (Ferron and Legget, 1994). Wright and Martin (1985) found similar RNA-DNA ratios (1 to 2 at 19–21°C) for starved striped bass, whereas fed striped bass larvae had ratios of 3–3.4 during the first two weeks after hatching. Robinson and Ware (1988) observed a similar trend in RNA-DNA ratios with starvation in the early life of larval Pacific herrings, as we did with red drum; ratios declined up to yolk-sac absorption, where the ratios leveled off. Values for RNA:DNA obtained in the laboratory in this study (1 to 2) were lower than previously reported values (2 to 4) for red drum larvae (Westerman and Holt, 1994).

As has been reported previously (Buckley, 1982; Ferron and Legget, 1994), the relation of growth rate and RNA:DNA changed with temperature. The higher mass-specific and protein-specific growth rates observed in the laboratory at 25°C, in comparison with those at 20°C and in the ponds at 32°C, as well as in comparsion with those at 25°C, were accompanied by lower RNA:DNA values (Tables 1 and 2). The inverse relation between RNA:DNA and temperature holds true in field-caught larvae as well. It was observed by Setzler-Hamilton et al. (1987), who found that in late spring, values for RNA-DNA ratios in striped bass larvae were higher than values measured in hotter, early summer months (spring values were about 3 and summer values were 2 to 2.5).

A high growth rate accompanied by a low RNA-DNA ratio, such as we observed in the 32°C ponds, is probably due to an increase in the efficiency of ribosomes in initiating protein synthesis and to an increase in the rate of chain elongation due to a direct effect of temperature, i.e., an increase in the production of protein per unit of ribosomal RNA due to a Q_{10} effect (cf. Westerman and Holt, 1988). Despite the effect of temperature on the relation of RNA:DNA and growth rate, RNA:DNA is a useful tool for determining nutritional status of fish larvae, particularly if it is understood that temperature contributes substantially to the relationship between RNA:DNA and growth (Buckley, 1982: Buckley et al., 1984; Ferron and Legget, 1994).

LDH Activity LDH, the terminal enzyme in vertebrate anaerobic glycolysis, is an important factor in the ability of some fish to produce sudden bursts of swimming and is found in large quantities in white muscle (Somero and Childress, 1980). The observed increase in LDH activity with age until death of starved larvae seems at first glance to conflict with priorities expected of an energy-deprived individual, in which metabolic processes would be expected to be declining. However, it is to be expected that LDH activity would be conserved, even in starving larvae, so that the muscle would remain functional as long as possible. A larva with no capability for movement would be doomed; thus, a metabolic investment in locomotory capability makes good adaptive sense.

Unlike in RNA:DNA, LDH activity showed a direct correlation with both mass- and protein-specific growth rate in the two fed laboratory treatments despite the increase in temperature from 20°C to 25°C. In the ponds, LDH activities showed an interaction with temperature similar to that seen in RNA:DNA, i.e. a lower specific activity at 32°C despite a higher growth rate. In the case of LDH, the declining activities observed in larvae from the higher temperature pond probably indicate that a lower concentration of enzyme is sufficient to maintain the catalytic efficiency needed by the tissues at the higher temperature (cf. Hochachka and Somero, 1984). The fact that a similar drop was not noted in the laboratory suggests a threshold for the drop in activity between 25°C and 32°C that was not present in the transition between 20°C and 25°C.

Clarke et al. (1992) found similar values for LDH in red drum larvae raised on wild zooplankton. Values for LDH activity in Clarke's study, assuming 87% water content, averaged 19–26 units/gWM for two-week-old larvae, slightly lower than the values we observed in the larvae raised in the laboratory and ponds.

Biochemical parameters as predictive tools

Although similar in their use as biochemical proxies for growth, LDH activity and RNA:DNA are fundamentally different in many other respects. RNA:DNA is a ratio of measured quantities, whereas LDH activity is a determination of a rate: a kinetic measurement. Inherent in the measurement of RNA:DNA is the assumption that the methods for determining the quantities of RNA and DNA are accurate, but there is no direct effect of temperature on the assay itself. For LDH, activities are measured in saturating conditions of substrate, which means that the activities are maximal activities (V_{max} from Michaelis-Menten kinetics; Lehninger, 1982) for each treatment. It is tacitly assumed that if assays are performed in saturating conditions at the same temperature, the differences in activity, or V_{max}, are due to differences in concentration of the enzyme. This assumption is

a reasonable one. It is important, however, to be aware of other potential causes of variability in the relation of both RNA:DNA and LDH activity to growth or condition in fish larvae. It has been demonstrated here and elsewhere (Ferron and Legget, 1994) that rearing temperature alters the relation of growth and biochemical proxies for growth. Another potential source of variability in the relation is the scaling of each of the proxies with individual size.

RNA:DNA increases slightly with individual size (Buckley,1982) but overall is insensitive to the changes in individual mass that would be expected in a study of larval fish growth within a single field sample. This is not the case for LDH activity which scales strongly with mass in fishes (e.g. Somero and Childress, 1980; Torres and Somero, 1988). In this study, a significant relation was observed between LDH activity (y, units/gWM) and protein mass (x, µg protein): $y = 2.25x^{0.187}$; P=0.02; r^2=0.43. RNA:DNA showed no significant change with size. Our study suggests that, for maximum accuracy, direct comparisons of field-caught larvae for LDH activity are best confined to narrow size ranges or the relation between LDH and size is described empirically. On the other hand, it could be argued that since mass-specific LDH activity increases with increasing mass, it is actually incorporating a growth-specific change within its scaling behavior, making it a better proxy. Either way, it shows considerable potential.

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Retrospective analysis of virtual population estimates for Atlantic menhaden stock assessment

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Abstract–Historical and retrospective comparisons of Atlantic menhaden virtual population analyses (VPA) from 1955 to 1995 revealed substantial inconsistency in estimates of management variables in the last year of stock assessments. Estimates of management variables from several historical stock assessments were generally consistent throughout most of the time series. In the last two years, however, historical estimates have deviated from revised estimates. Relative performance of alternative ad hoc methods for estimating fully recruited fishing mortality (F) in terminal years showed that all methods were imprecise, but conventional catch-curve estimates were unbiased and had the least retrospective inconsistency. Retrospective differences in terminal estimates of age-1 F by separable VPA ranged widely for eight alternative settings but were clearly minimized by using seven years of catch data. The general magnitude of retrospective difference was ±1.2 billion recruits (46% relative difference), ±9,000 metric tons of spawning stock biomass (33% relative difference), and ±4.7 percent maximum spawning potential (106% relative difference). Retrospective differences in recruitment, spawning stock biomass, and spawning potential were positively skewed but not biased, indicating that the frequency of positive and negative inconsistencies are equal but that the positive differences are much greater in magnitude. The skewed distribution of retrospective inconsistency should be considered for managing the Atlantic menhaden fishery.

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The Atlantic menhaden, *Brevoortia tyrannus*, is a planktivorous clupeid that schools in coastal waters off the east coast of the United States. Atlantic menhaden dominated total U.S. fishery landings from 1946 to 1962 (Ahrenholz et al., 1987), yielding approximately 600,000 metric tons (t) per year 1953–62 (Henry, 1971), after which landings steadily declined to 162,000 t in 1969 owing to recruitment failure and subsequent overfishing. Since then, catch has increased to an average of 330,000 t per year 1970–95 (AMAC, 1992).[1] The Atlantic menhaden fishery is currently managed according to six fishery and population thresholds that indicate overfishing in relation to historic production (AMAC, 1992). Three of the minimum population thresholds (2 billion recruits, 17,000-t spawning stock biomass, and 3% maximum spawning potential) are derived from virtual population analysis (VPA) (Megrey, 1989).

Atlantic menhaden landings have been reported from processing plants since 1940 and have been sampled for length, weight, and age since 1955 according to a two-stage cluster sampling design in which fish were sampled weekly from each port where menhaden were processed (Nicholson, 1975; Chester, 1984; Smith et al., 1987). The frequency of fishery samples and the consolidated nature of the fishery provide an extremely reliable 41-year series of catch at age, ages $0–6^{+}$, for estimation of abundance and mortality through VPA (Table 1). Unfortunately, no independent indices of relative abundance are available to calibrate abundance estimates for the last year of catch: commercial catch per unit of effort is a biased index because commercial catchability is inversely related to abundance (Schaaf, 1975; Ahrenholz et al., 1987; Vaughan and Smith, 1988; Atran and Loesch, 1995) and fishery-independent survey indices are not correlated with abundance (Ahrenholz et al., 1989). In the absence of reliable abundance indices, and therefore of a formal statistical estimator for year-class abundance in the last year of the VPA, ad hoc estimation rules have been used to approximate abundance.

The error in estimates of abundance is progressively less in previous years than in the last year of

[1] 1992–95 landings from Joseph Smith, Beaufort Laboratory, National Marine Fisheries Service, Beaufort, NC. Personal commun.

Table 1

Age-based stock assessments of Atlantic menhaden. Y_t indicates the terminal year the VPA.

Y_t	Source
1976	AMMB, 1981; Powers, 1983
1981	Vaughan et al., 1986; Ahrenholz et al., 1987
1984	AMMB, 1986; Vaughan and Smith, 1988
1988	Vaughan, 1990; Vaughan and Merriner, 1991
1990	AMAC, 1992 (p. 40–50); Vaughan, 1993
1992	AMAC, 1992 (p. 17–30)
1993	Vaughan, 1994[1]
1994	Vaughan, 1995[1]
1995	Vaughan, 1996[1]

[1] Vaughan, D. S. Trigger variables for Atlantic menhaden. Natl. Mar. Fish. Serv., NOAA, Unpubl. AMAC reports.

the VPA, provided that catch at age and natural mortality (M) are well estimated and fishing mortality (F) is at least moderate (Jones, 1961; Tomlinson, 1970; Pope, 1972; Ulltang, 1977; Megrey, 1989). As stated in the Atlantic menhaden fishery management plan, "Trigger estimates for recent years from VPA are subject to large uncertainty, while estimates 2 to 3 years old are more reliable" (AMAC, 1992). Consistency in successive stock assessments can be evaluated by using "historical analysis," which compares estimates from the most recent assessment with contemporary estimates from prior stock assessments (Sinclair et al., 1985),[2] but historical assessments of the menhaden stock were not conducted with a common estimation rule. Consistency of the current estimation rule can be evaluated by using "retrospective analysis," which recreates a historical series of VPA's with a single estimation rule (Sinclair et al, 1990).[2]

The first objective of the current investigation was to report the general magnitude and potential bias of retrospective differences for guidance on interpreting current estimates and for providing fishery management advice. The second objective was to attempt alternative estimation rules to improve consistency of estimates.

[2] Examples of historical and retrospective analyses, interpretation, and discussion can also be found in the following two refererences:

Int. Counc. Explor. Sea. 1991. Report of the working group on methods of fish stock assessments. ICES Council Meeting Assess., p. 25.

Northeast Fisheries Science Center. 1994. Report of the 18th Northeast Regional Stock Assessment Workshop (18th SAW). NEFSC Ref. Doc. 94-22.

Methods

Historical comparisons

Three Atlantic menhaden management variables derived from VPA (age-1 abundance [R], spawning stock biomass [SSB], and percent maximum spawning potential [%MSP]) were compared among ten reported stock assessments (Table 1). The number of historical estimates of each variable differed because some reports did not document all three population estimates.

Consistency of successive stock assessments was measured by comparing historical estimates with revised estimates (from the 1995 VPA), which are more reliable. Inconsistency may result from historical estimation error or inaccurate estimates for prior years in the current VPA (Sinclair et al., 1990). The population thresholds used to define overfishing are subject to some uncertainty because they are also VPA estimates; but they are converged estimates, which are much more certain than current estimates. In comparing current VPA estimates with these overfishing thresholds for an annual assessment of stock status, converged and current estimates are assumed to be consistent. Estimates in the last year (Y_t), and back-calculated years (Y_{t-1}, Y_{t-2}, etc.) were compared with the time series of estimates derived in 1995. Differences between historical estimates and revised estimates were calculated as follows:

$$\Delta R_{t,t+k} = \mathrm{R}_{t,t+k} - \mathrm{R}_{t,1995},$$

where $R_{t,1995}$ = the most recent estimate of recruitment in year t;

$R_{t,t+\mathrm{k}}$ = recruitment in year t as estimated when $t+k$ was the last year in the assessment; and

k = is the retrospective lag between year t and the last year of the historical VPA.

For example, $R_{1990,1993}$ is the 1993 estimate of 1990 recruitment, which has a three-year retrospective lag (i.e. k=3). When k = 0, $R_{t,t}$ is an estimate of recruitment for the last year in an assessment and is referred to as a terminal estimate. Historical differences in SSB and %MSP were similarly calculated:

$$SSB_{t,t+k} = SSB_{t,t+k} - SSB_{t,1995}$$
$$\Delta\%MSP_{t,t+k} = \%MSP_{t,t+k} - \%MSP_{t,1995}.$$

Root mean square (RMS) difference was used as a measure of dispersion of historical estimates from

converged estimates for the additive properties of mean square difference. Sample sizes for historical differences were low, because of the limited number of historical stock assessments, but the following retrospective analyses have greater sample size for estimating RMS difference (n>30).

Retrospective comparisons

Retrospective analysis was performed in two stages to investigate consistency of both elements of the estimation rule: 1) estimation of fully recruited F by ad hoc methods and 2) estimation of partial recruitment to the fishery at ages 0 and 1 by separable VPA (SVPA; Pope and Shepherd, 1982). Both analytical stages assumed that menhaden were fully recruited to the fishery at age 2 and that M was 0.45 for all ages, over the entire time period.

Fully recruited F was approximated by using three alternative ad hoc methods for the first element of the analysis. Conventional catch curves (Beverton and Holt, 1957; Ricker, 1975; Gulland, 1983) and modified catch curves (Chapman and Robson, 1960; Robson and Chapman, 1961) were used to estimate mortality of the age-5 cohort over the four terminal years of the catch record (i.e. ages 2–5). These two catch-curve methods assumed that F in the current year was similar to F experienced by that cohort over the previous three years. The third ad hoc method, log catch ratios (Ricker, 1975; Gulland, 1983), derived fully recruited F from the negative log ratio of age-3$^+$ abundance in the terminal year to age-2$^+$ abundance in the previous year and assumed that F in the last year was similar to F in the previous year. All three ad hoc methods assume that menhaden are fully recruited and equally available to the fishery at age-2$^+$, which was confirmed through inspection of back-calculated F from the 1995 VPA.

The second element of the assessment, estimation of partial recruitment, was performed by using SVPA on a fixed number of years. For example, a retrospective series of 5-year SVPA's was produced with the following algorithm.

Step 1 SVPA was run on an initial time series of catch-at-age data (e.g. 1955–60) with the appropriate estimate of fully recruited F in the terminal year.

Step 2 Catch data in the starting year (e.g. 1955) were deleted, and catch data from a new terminal year (e.g. 1961) were appended.

Step 3 SVPA was rerun on the revised time series with the appropriate estimate of fully recruited F in the new terminal year.

Steps 2 and 3 were repeated until 1995 was the terminal year.

Significance of retrospective bias was tested with a conventional t-ratio test (H_0: mean difference=0). Normality was tested by using the Shapiro and Wilk (1965) method. Results of t-ratio tests were confirmed by using nonparametric chi-square and sign tests. Dispersion of retrospective estimates from converged estimates was compared among alternative assessment rules by using RMS of retrospective differences. Retrospective estimates of fully recruited F in terminal years were compared with back-calculated estimates of fully recruited F from the 1995 VPA, as the average of ages 2–5 (weighted by abundance), to derive retrospective differences:

$$\Delta F_{t,t} = F_{t,t} - F_{t,1995}.$$

Note that there is no k subscript, as there were in the formulae for historical comparisons, because all retrospective estimates of fully recruited F were for terminal years (i.e. k=0). The relative retrospective difference ($\Delta F_{t,t}/F_{t,1995}$) was also calculated to remove the magnitude of the estimate from estimates of general inconsistency.

Back-calculated estimates of fully recruited F from the 1995 VPA were used in terminal years to compare retrospective inconsistency of SVPA settings without including retrospective inconsistency from ad hoc estimates of terminal F. The fixed number of years in each series of retrospective SVPA's was varied from three to ten years by using the algorithm described for the five-year example above. Retrospective consistency was compared among the eight series of retrospective SVPA's according to RMS difference of age-1 F estimates. Full recruitment of age- 2 and oldest age (6$^+$) menhaden was confirmed through inspection of back-calculated F at age from the 1995 VPA and was not adjusted for retrospective comparison.

Final SVPA runs were performed with seven years of catch-at-age and catch-curve estimates of terminal F to emulate more realistic inconsistency and describe the general magnitude and direction of retrospective differences. Retrospective estimates of R were derived directly from SVPA terminal estimates of age-1 abundance. SSB was estimated from terminal SVPA estimates of age-3$^+$ abundance and estimated weight at age of spawners. Percent MSP was calculated according to egg production per recruit (Vaughan, 1990; AMAC, 1992). Retrospective differences and relative differences of management variable estimates were log transformed [e.g. log$_e$ (R+ constant)] to test bias, and geometric mean square was used to estimate mean square difference because differences had skewed distributions.

Results

Historical comparisons

Management variable estimates from past stock assessments were generally consistent throughout most of the time series, except for the last two years of each assessment, when some historical estimates deviated from revised estimates from the 1995 VPA (Fig. 1). Terminal estimates of age-1 abundance were greater than revised estimates for five assessments and less than revised estimates for three assessments, but positive historical differences (i.e. historical estimate > revised estimate) were greater. For ex-

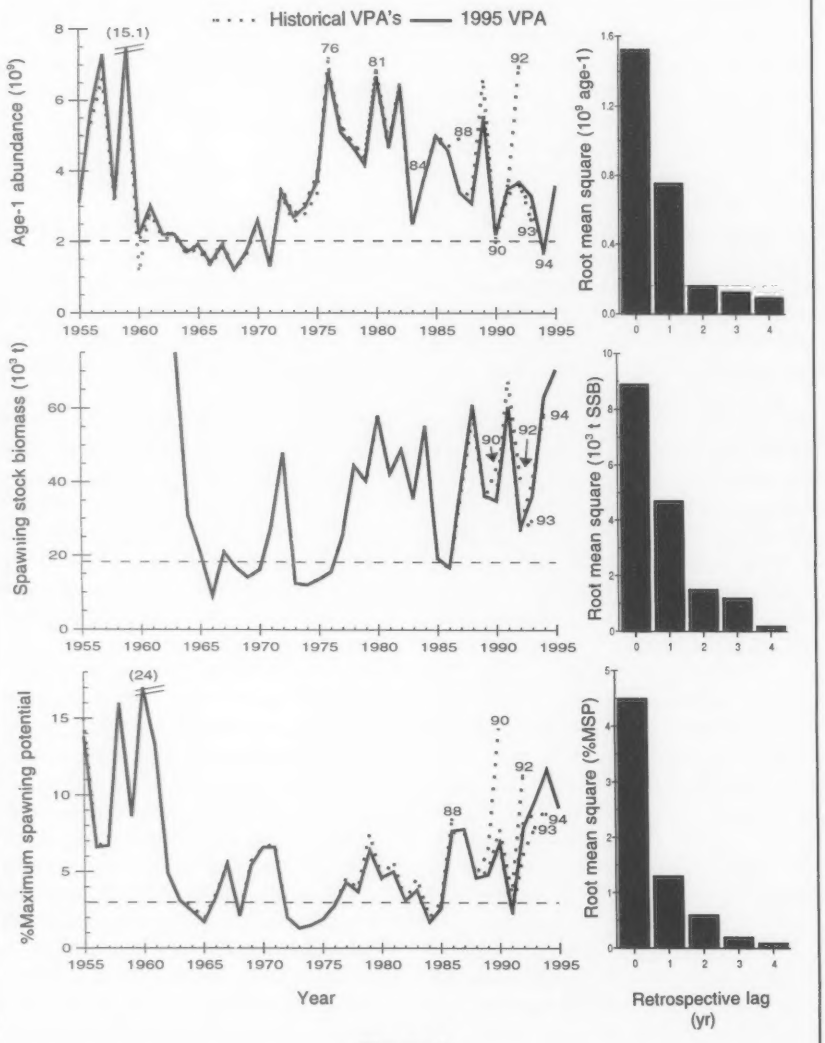

Figure 1

Comparison of historical estimates of Atlantic menhaden recruitment, spawning stock biomass, and percent maximum spawning potential. In the left charts, terminal years of historical stock assessments are labeled at the end of each series, overfishing thresholds are indicated by broken horizontal lines, and values in parentheses are not plotted. Convergence of estimates is illustrated by reduction in root mean square of historical differences over time in the charts on the right.

ample, in 1992, recruitment was estimated to be 3.4 billion greater than the 1995 estimate of 1992 recruitment. Estimates converged to within 160 million recruits of 1995 VPA estimates, when the retrospective lag (k) was greater than one year.

Only five terminal estimates of SSB and %MSP were available, including 1995 estimates. Two historical estimates of SSB were greater than revised estimates and two were less than revised estimates. Historical estimates of SSB converged to within 1,500 t of 1995 VPA estimates when k>1 year.

Historical estimates of %MSP were greater than revised estimates for three assessments and less than revised estimates for two assessments. The 1992 estimate of 1991 %MSP (i.e. backcalculated one year) was 3.6, but subsequent estimates of 1991 %MSP were below the overfishing threshold of 3 %MSP. Therefore, an overfishing trigger fired, but it was not detected until two years later. Estimates of %MSP converged to within 0.6 of 1995 VPA estimates when k>1 year.

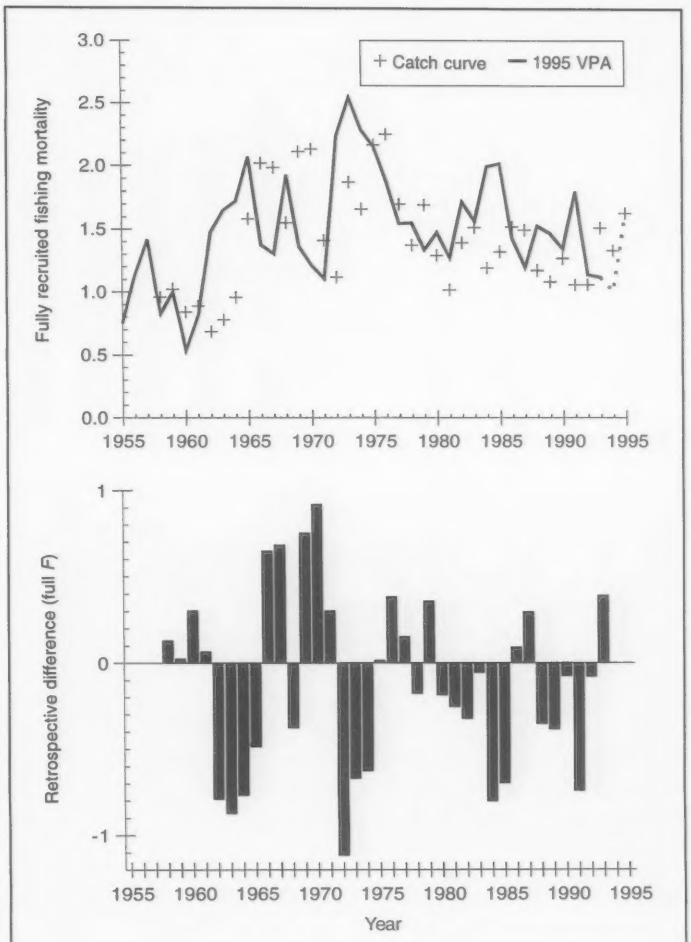

Figure 2

Retrospective catch-curve estimates of Atlantic menhaden fishing mortality and back-calculated estimates from the 1995 VPA (above) and retrospective differences (below). The broken line in the upper chart indicates provisional estimates.

Retrospective comparisons

Conventional catch curves produced the most consistent estimates of fully recruited F among the three ad hoc methods used. Retrospective differences from catch-curve estimates in terminal years ranged from –1.12 to 0.92 (Fig. 2). The RMS difference was 0.51 and the RMS relative difference was 33% (n=36). The mean retrospective difference in fully recruited F was not significantly different from zero. Therefore, although terminal estimates were imprecise, they were not biased. Retrospective differences in catch-curve estimates were negatively correlated with back-calculated F from the 1995 VPA (r=–0.62)(i.e. when F was low, catch-curve estimates were generally greater than revised estimates; when F was high, catch-curve F was generally less than the revised estimate). Retrospective differences in fully recruited F produced opposite inconsistencies in SSB and %MSP. For example, in 1993, catch-curve F was greater than the revised F (Fig. 2), and initial VPA estimates of SSB and %MSP were less than revised estimates (Fig. 1). Alternative methods of estimating fully recruited F produced even greater inconsistency. The RMS retrospective difference from modified catch curves was 0.60 (49% relative difference), and log catch ratios produced a RMS difference of 0.61 (52% relative difference) (n=36 for both methods).

Retrospective differences in estimates of age-1 F from SVPA ranged from –0.59 to 0.45 for all retrospective SVPA's and were not significantly different from zero (n=31 for each series). RMS difference was minimized when seven years of catch-at-age data were used and increased regularly as the number of years deviated from seven (Fig. 3). The RMS retrospective difference for estimates of age-1 F was

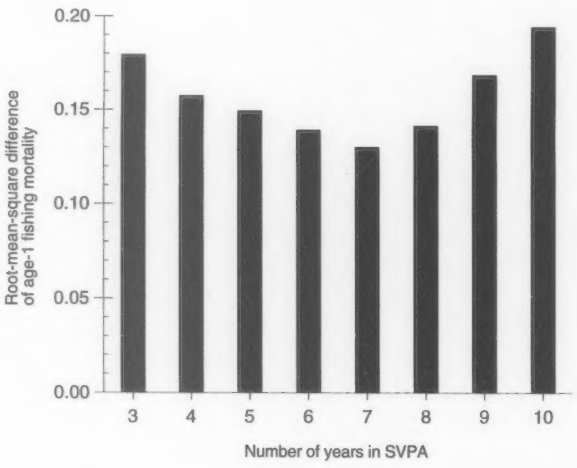

Figure 3

Root-mean-square retrospective difference of fishing mortality estimates for age-1 Atlantic menhaden from SVPA's with three to ten years of catch at age.

0.13 (33% relative difference) with back-calculated values of fully recruited F and increased to 0.18 (45% relative difference) with catch-curve estimates (Fig. 4).

Retrospective differences in age-1 abundance ranged from –2.4 billion to 11.5 billion individuals in terminal years (Fig. 5). There was no significant bias in log-transformed differences, and the RMS difference was 1.2 billion recruits (n=34). The RMS relative difference for estimates of age-1 abundance was 46%.

Retrospective differences in terminal SSB estimates ranged from –72,000 t to 448,000 t (Fig. 6). The large positive differences in 1962 and 1963 SSB were primarily due to large negative differences in fully recruited F (Fig. 2). Log-transformed retrospective differences were not significantly biased, and the RMS difference was 9,000 t SSB (n=34). The RMS relative difference for estimates of SSB was 33%. Retrospective differences in %MSP ranged from –5.5 to 19.5 in terminal years (Fig. 7).

The RMS difference was 4.7 %MSP, and there was no significant bias in log-transformed differences (n=34). The RMS relative difference for estimates of %MSP was 106%, because inconsistencies were larger than the estimated level of %MSP. Retrospective differences in %MSP were negatively correlated with retrospective differences in fully recruited F (r=–0.79).

Discussion

This case study illustrates how retrospective comparisons can provide useful data for analytical decisions and reveal important insights for management advice, especially in situations where statistical estimates of uncertainty are not available. For example, SVPA with seven years of catch data clearly provided more consistent results than SVPA of longer or shorter time series (Fig. 3). A time period of seven years appears to be long enough to smooth annual variation in partial recruitment, while including only years which represent the current schedule of F at age. By including more years in the analysis, there is a likelihood that catches from substantially different exploitation patterns will be incorporated. Performance of alternative intervals of catch data was judged according to general conditions over three decades. Although such guidance is valuable, specific SVPA runs should be examined to confirm the assumption of separability. For example, targeting specific cohorts, such as the superabundant 1958 year class, may change fishing patterns. Abrupt changes should be reflected in patterns of log catch-ratio residuals (Pope and Shepherd, 1982) and may necessitate a longer or shorter time series of catch at age for SVPA.

Retrospective inconsistency can result from a host of systematic problems, including errors in the cur-

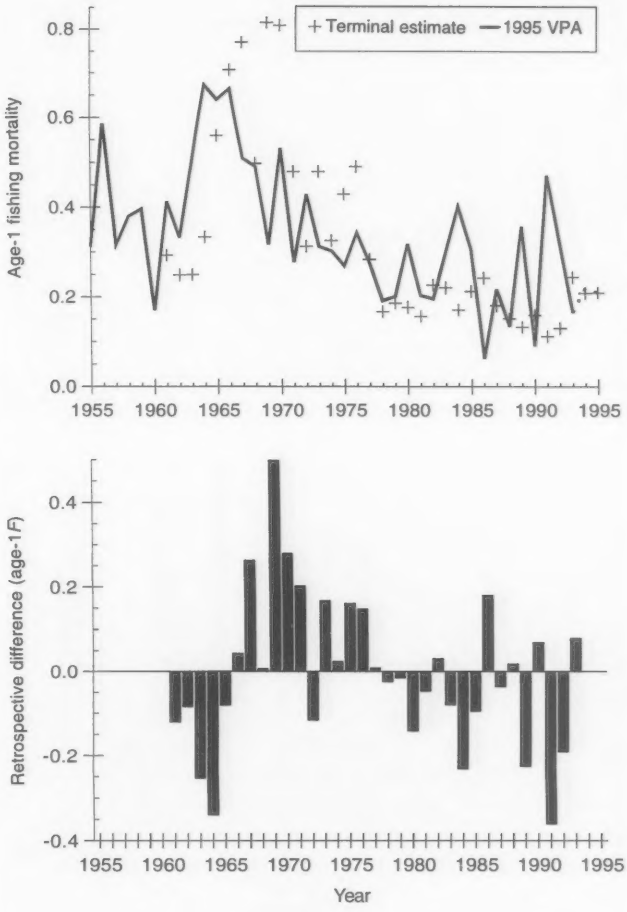

Figure 4

Estimates of fishing mortality for age-1 Atlantic menhaden from the 1995 VPA and terminal estimates from retrospective SVPA's (above) and retrospective differences (below). The broken line in the upper chart indicates provisional estimates.

rent VPA (Sinclair et al., 1990). For example, Lapointe et al. (1989) simulated Atlantic menhaden catch for VPA's to show that 50% underestimates of M produced 12% overestimation of recruitment and 6% overestimation of biomass and that overestimation of M produced similar underestimates of recruitment and biomass. Therefore, the assumption that M is constant when M varies among years and ages may cause VPA inconsistency. It appears that the inability to estimate fully recruited F accurately in terminal years accounts for a substantial portion of the retrospective differences in management variables reported here. Inaccurate estimates of terminal F may result from abrupt changes in F or M. Catch-curve estimates of terminal F are not sensitive to changes in current mortality because they reflect the average mortality over the last three years more than mortality in the terminal year.

Retrospective differences in management variables were positively skewed, and log transformation was necessary to test for bias. Skewed retrospective differences with no bias imply that positive and negative differences occur with equal frequency, but positive differences are generally greater in magnitude. Theoretically, normally distributed errors in F will produce lognormally distributed errors in R,

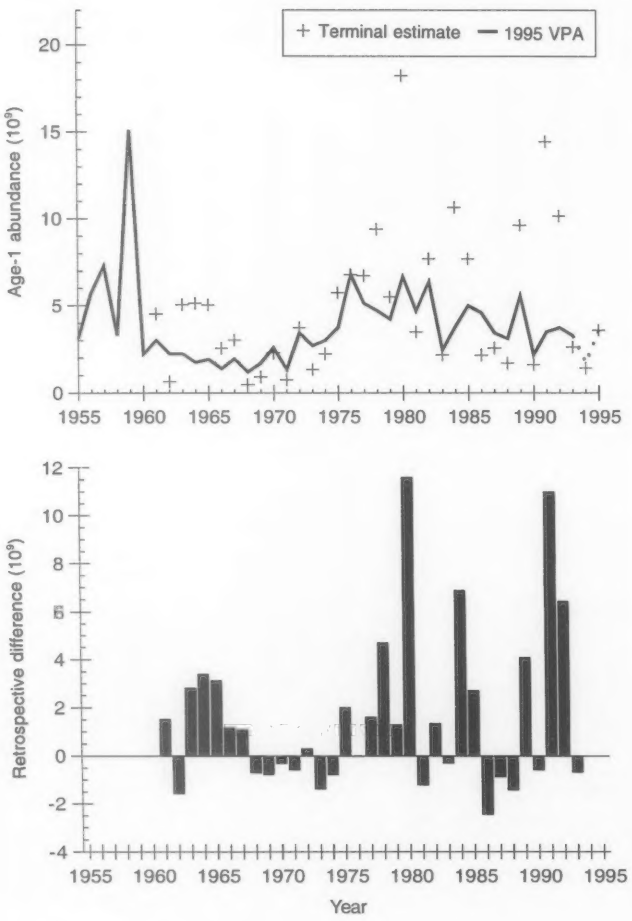

Figure 5

Estimates of Atlantic menhaden recruitment from the 1995 VPA and terminal estimates from retrospective SVPA's (above) and retrospective differences (below). The broken line in the upper chart indicates provisional estimates.

SSB, and %MSP because they are based on estimated abundance, biomass, and egg production, respectively, which have a negative exponential relation with fishing mortality. Therefore, small underestimates in F can produce large overestimates of abundance. Retrospective differences may also be skewed because negative values of R, SSB, and %MSP are not possible. Despite the conclusion that log-transformed retrospective differences were not biased, the positive skewness of retrospective differences and relative differences has important implications for management of the fishery. A skewed distribution of inconsistency from converged estimates may be considered a characteristic feature of terminal estimates from future menhaden VPA's. Therefore, management advice should account for the equal likelihood of moderate underestimation and substantial overestimation of R, SSB, and %MSP.

Management decisions must consider the difference between converged estimates, which are used to define overfishing thresholds, and terminal estimates, which are used to determine current status. Although inconsistency does not quantify uncertainty of estimates, the large retrospective differences re-

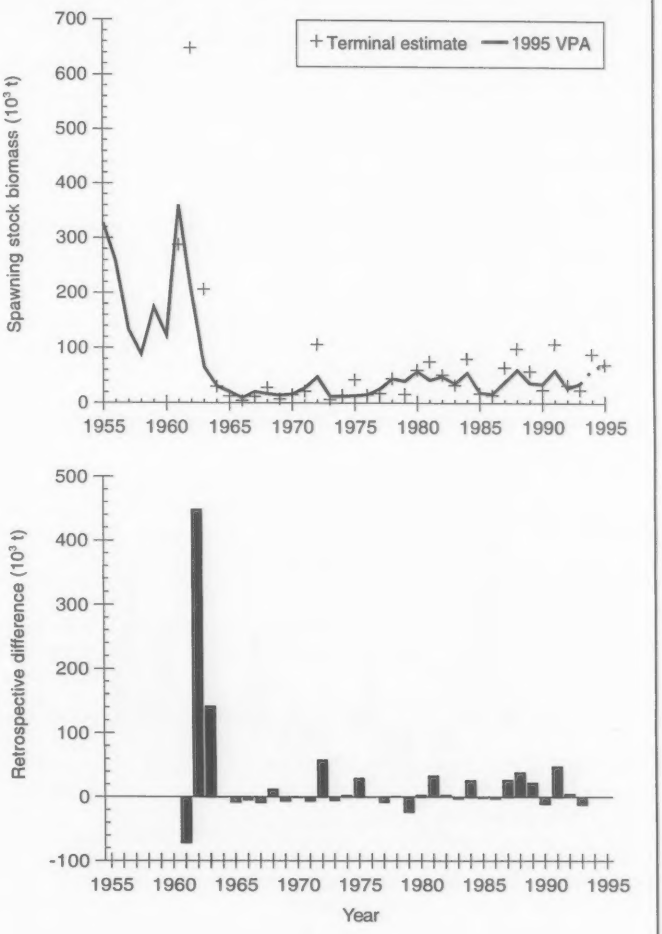

Figure 6

Estimates of spawning stock biomass of Atlantic menhaden derived from the 1995 VPA and terminal estimates from retrospective SVPA's (above) and retrospective differences (below). The broken line in the upper chart indicates provisional estimates.

ported here suggest large uncertainty in terminal estimates. Fredrick and Peterman (1995) simulated uncertainty in Atlantic menhaden VPA's to show that much more conservative overfishing thresholds would be needed to incorporate high levels of uncertainty into risk-averse management decisions. An alternative to incorporating uncertainty adjustments is to make management decisions based on converged estimates that indicate conditions of two years earlier (AMAC, 1992). If more timely management is desirable, methods to calibrate terminal estimates of abundance are necessary.

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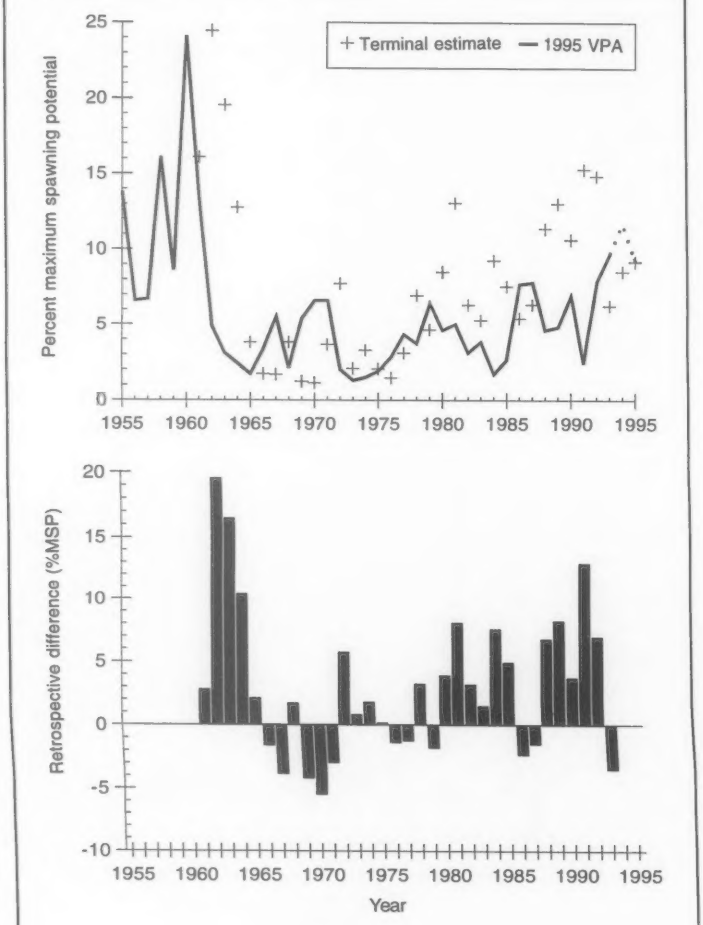

Figure 7

Estimates of percent maximum spawning potential of Atlantic menhaden derived from the 1995 VPA and terminal estimates from retrospective SVPA's (above) and retrospective differences (below). The broken line in the upper chart indicates provisional estimates.

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Maturation and reproductive seasonality in bonefish, *Albula vulpes*, from the waters of the Florida Keys

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Abstract.—We examined 528 bonefish to estimate length and age at sexual maturity and to describe seasonal patterns in gonadal development. These fish ranged from 21 to 702 mm fork length (FL) and were collected in South Florida waters from 1989 to 1995. Gonads of 437 bonefish were examined histologically, and gonadosomatic indices (GSI) were calculated for 449 bonefish. Male bonefish reached 50% sexual maturity (the predicted size and age at which half the individuals are expected to be sexually mature) at 418 mm FL (95% confidence interval 393–443 mm) and an age of 3.6 years (95% confidence interval 3.3–3.9 years). Females reached 50% sexual maturity at 488 mm FL (95% confidence interval 472–504 mm) and 4.2 years (95% confidence interval 3.9–4.6 years). Lengths and ages at 50% maturity for males and females were significantly different. The smallest sexually mature male was 425 mm FL, and the smallest sexually mature female was 358 mm FL. The youngest sexually mature male was 3 years old, and the youngest sexually mature female was 2 years old. Gonadal activity was seasonal and peaked during November–May. Vitellogenic oocytes were present in ovaries in every month except August and September and were most abundant during November–May. Median GSI's were greatest during November–May and least during July–September for both males and females. No fully hydrated ovaries or postovulatory follicles were found, therefore we could not estimate spawning periodicity or batch fecundities. Total fecundity ranged from 0.4 to 1.7 million oocytes and had a significant positive relation to fish weight. The absence of fully hydrated ovaries and postovulatory follicles in the bonefish we sampled suggests that bonefish spawn outside the traditional shallow-water (<2 m) fishing grounds in the Florida Keys.

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Bonefish, *Albula* spp., frequent coastal and inshore waters of tropical seas worldwide and are the basis of economically important recreational fisheries in many areas of their range. Although 23 nominal *Albula* species have been described (Whitehead, 1986), only two Atlantic species, *A. vulpes* and *A. nemoptera*, are recognized (Rivas and Warlen, 1967). In the western Atlantic, *A. vulpes* is common in the Florida Keys, the Bahama Islands, and throughout the Caribbean Sea (Hildebrand, 1963). *Albula nemoptera* appears to have a more restricted distribution than *A. vulpes* and has been reported from the Guianas, Venezuela, Columbia, Panama, Jamaica, and Hispaniola (Rivas and Warlen, 1967; Uyeno et al., 1983). The single record of *A. nemoptera* from Florida waters is considered questionable (Robins and Ray, 1986), and the species has not been reported from the Bahama Islands (Böhlke and Chaplin, 1993).

Bonefish are esteemed for their wariness and fighting abilities, and fishing for them provides an important source of income to Florida Keys and Bahamian fishing guides. Commercial sale of bonefish in Florida is prohibited, and regulations on the recreational fishery restrict catches to one fish per angler per day and the length of captured fish to a minimum total length of 457 mm (390 mm fork length). Most bonefish caught in Florida waters are released.

The life history of bonefish has not been adequately described. Crabtree et al. (1996) described the age and growth of bonefish from South Florida and found that bonefish can attain ages of 19 years. Female and male growth models were significantly different; females were slightly longer than males of the same age. Although the age and growth of Florida Keys bonefish have been studied, important questions remain regarding bonefish reproduction. Bruger (1974) reported sexually mature females as small as 210 mm standard length (221 mm fork length) and as young as 1 year from waters off the Florida Keys, but his sample size was inadequate to determine the age or length at 50% maturity (the predicted size and age at which half the individuals are expected to be sexually mature). Bruger found ripe female bonefish throughout the year in waters off the Florida Keys and concluded that reproduction was not seasonal, but his sample size was small (n=148) and his conclusions equivocal. In other areas, bonefish reproduction appears to be seasonal according to patterns of larval and juvenile abundance (Alexander, 1961; Pfeiler, 1984; Pfeiler et al., 1988; Mojica et al., 1995). There

is also no published information on bonefish fecundity. In this article, we estimate the age and length at which sexual maturity is attained and describe the seasonal cycle of gonadal development in bonefish from waters off the Florida Keys. We also estimate the total fecundity of 33 bonefish collected from these waters.

Methods

Sampling

We examined 528 bonefish collected from South Florida waters from February 1989 to April 1995. Most of these bonefish were caught with hook-and-line gear either by biologists or by a single professional bonefish guide and his anglers from waters off the Florida Keys and in Florida and Biscayne Bays. Five bonefish caught with hook-and-line gear were obtained from taxidermists in Fort Lauderdale and five others from tournaments in the Keys. Supplemental collections of small bonefish (<425 mm) were made with various-size seines and gill nets in waters off the Keys. Ages, based on validated sectioned otoliths and growth rates of these bonefish, were described by Crabtree et al. (1996).

Fork length (FL) was measured to the nearest millimeter (mm), and fish were weighed to the nearest gram. Sex, gonad condition, and gonad weight (g) were recorded. Gonad samples for histology and for estimation of fecundity were removed from the fish and preserved in 10% buffered formalin; they were later soaked in water for one hour and then stored in 70% ethanol. Histological sections of gonads from 437 bonefish ranging from 228 to 702 mm were prepared and assessed for reproductive state. Gonad samples were processed histologically with a modification of the periodic acid Schiff's (PAS) stain for glycol-methacrylate sections, with Weigert's iron-hematoxylin as a nuclear stain and metanil yellow as a counterstain (Quintero-Hunter et al., 1991).

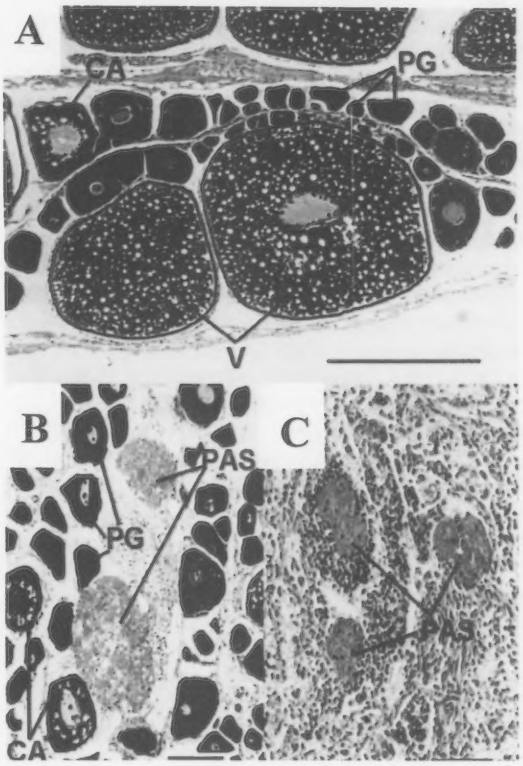

Figure 1

(**A**) A histological section from an ovary of a 677-mm-FL bonefish, *Albula vulpes*, showing oocyte stages. PG = primary growth oocytes, CA = cortical alveolar oocytes, and V = vitellogenic oocytes. Scale bar = 400 microns. (**B**) A histological section showing PAS-positive melanomacrophage centers (PAS), cortical alveolar oocytes, and primary growth stage oocytes in a regressed ovary from a 692-mm-FL bonefish. When stained with periodic acid Schiff's stain, melanomacrophage centers are brilliant purple. Scale bar = 100 microns. (**C**) A histological section showing PAS-positive melanomacrophage centers in a regressed testis from a 586-mm-FL bonefish. Scale bar = 50 microns.

Oocyte staging

Oocytes were staged and counted from histological preparations at 100× with a compound microscope attached to a digital image-processing system. Three oocyte stages were recognized in bonefish ovaries: primary growth, cortical alveolar, and vitellogenic (Wallace and Selman, 1981; Fig. 1A). In addition, we counted PAS-positive melanomacrophage centers (Ravaglia and Maggese, 1995), which were present in many ovaries (Fig. 1B). When stained with the PAS stain, these structures are brilliant purple. Melanomacrophage centers are thought to be active in degrading atretic oocytes, postovulatory follicles, and residual cells of the spermatogenic cycle (Chan et al., 1967; Ravaglia and Maggese, 1995). At least 300 combined oocytes and melanomacrophage centers per slide were staged and counted in arbitrarily chosen fields, and frequencies were expressed as a percentage of the total count. We counted all structures that had at least 50% of their area visible in a field before moving to the next field. The presence of atretic hydrated oocytes was also noted.

Length and age at sexual maturity

Females were considered sexually mature if vitellogenic oocytes were present or if the histological sections appeared disorganized, highly vascularized, and contained widespread evidence of atresia. Documentation of atresia followed the classification of Hunter and Macewicz (1985). Immature females had small (gonadosomatic index <0.35), well-organized gonads that contained little evidence of atresia. We interpreted the widespread occurrence of PAS-positive melanomacrophage centers in inactive ovaries as evidence of past gonadal development, and we considered bonefish that had regressed (no vitellogenic oocytes present) ovaries containing many of these structures to be sexually mature (Fig. 1B). Males were considered sexually mature if the testes contained evidence of ongoing spermatogenesis, residual sperm, or widespread PAS-positive melanomacrophage centers associated with gonadal recrudescence (Fig. 1C).

Sometimes distinguishing between the gonads of sexually immature bonefish and the regressed gonads of mature fish was difficult. We reduced the probability of misclassifying regressed and immature fish by eliminating all bonefish collected during June–October from our analyses of age and length at sexual maturity. June–October was the season of minimal gonad development in bonefish, and most of the regressed bonefish in our sample were captured during this period. By excluding the postreproductive months from our analysis, we eliminated 84% of the regressed females and 63% of the regressed males in our sample. Hunter et al. (1992) recommended that only fish collected early in the spawning season be used to estimate the length at 50% maturity, but our sample size was not large enough to allow us to restrict our analysis to this extent.

To describe age and length at sexual maturity, we used nonlinear regression procedures to determine the inflection point of a logistic function fitted to the percentage of males and females that were sexually mature and to their respective lengths and ages. Parameter b in Table 1 is the inflection point and is the estimate of length or age at 50% maturity. Likelihood-ratio tests were used to compare the overall regression models and parameter estimates for males and females (Kimura, 1980).

Seasonality of gonad development

Monthly median gonadosomatic indices (GSI) of sexually mature males and females were plotted to show seasonal reproductive patterns. GSI's were calculated for 449 bonefish ranging from 228 to 702 mm as

$$GSI = (GW/(TW - GW)) \times 100,$$

where GW = total gonad weight (g); and
TW = total fish weight (g).

We also plotted the monthly frequency of occurrence of the various oocytes stages that we counted for fish

Table 1

Percentage mature-age, percentage mature fork length, and weight-fecundity regressions for bonefish, *Albula vulpes*, from the waters of the Florida Keys. Wt = weight (g), FL = fork length (mm), AGE = age in years, FEC = fecundity. Values in parentheses are standard errors.

Y	X	n	a (1 SE)	b (1 SE)	r^2	Range of X for regressions
			$Y = \left(1/\left(1+e^{(-a(X-b))}\right)\right) \times 100$			
% Mature (Females)	FL	150	0.028 (0.0064)	487.6 (8.14)	0.632	228–702
% Mature (Females)	AGE	143	1.122 (0.2345)	4.24 (0.192)	0.445	1–19
% Mature (Males)	FL	116	0.545 (2.8227)	417.5 (12.59)	0.735	322–687
% Mature (Males)	AGE	109	1.618 (0.3674)	3.60 (0.156)	0.464	2–19
			$Y = a + bX$			
$\log_{10}$FEC	$\log_{10}$Wt	33	1.936 (0.4708)	1.131 (0.1312)	0.706	1,790–5,790

collected in the years during which we had regular monthly collections.

Fecundity

The total fecundity (the standing stock of advanced yolked oocytes) of 33 bonefish was estimated gravimetrically. Ovaries were subsampled from anterior, middle, and posterior portions of each ovary to evaluate spatial variations in oocyte size within the ovary and between ovaries. Subsamples of ovary containing 1,000–1,500 vitellogenic oocytes were weighed to the nearest 0.01 mg, and total fecundity was calculated on the basis of the mean number of oocytes per gram of ovary. Ovaries that contained widespread atresia, which suggested that partial spawning might have occurred, were not used for fecundity estimation.

Results

Two of the bonefish that we examined were statistically significant outliers (Crabtree et al., 1996); both were exceptionally small for their estimated ages and the weights of their otoliths were exceptionally light. Crabtree et al. excluded both fish from growth models, age-frequency distributions, and otolith weight-age regressions, and we also excluded them from our analyses. One was a 351-mm female that was 7 years old and the other was a 458-mm female that was 18 years old. Both fish were caught with hook-and-line gear on the ocean (Florida Straits) side of North Key Largo, and they were the smallest females examined whose ovaries contained vitellogenic oocytes. The 458-mm female had oocytes that were in the nuclear migratory stage, and these were the most advanced oocytes we found in any bonefish.

Length and age at sexual maturity

Male bonefish reached 50% sexual maturity at a length of 418 mm (95% confidence interval 393–443 mm) and an age of 3.6 years (95% confidence interval 3.3–3.9 years); females reached 50% sexual maturity at a length of 488 mm (95% confidence interval 472–504 mm) and an age of 4.2 years (95% confidence interval 3.9–4.6 years; Fig. 2; Table 1). The lengths at 50% maturity (χ^2=124.43, df=1, P<0.001) and the ages at 50% maturity (χ^2=5.59, df=1, P=0.018) for males and females were significantly different. In addition, the overall logistic equations for length at 50% maturity (χ^2=51.18, df=2, P<0.001) and for age at 50% maturity (χ^2=11.55, df=2, P=0.003) for males and females were significantly different. The smallest sexually mature male was 425 mm long, and the smallest sexually mature female was 358 mm long. The youngest sexually mature male was 3 years old, and the youngest sexually mature female was 2 years old. All males longer than 477 mm and all females longer than 594 mm were sexually mature. All males older than 5 years and all females older than 7 years were sexually mature.

Primary growth stage oocytes were present in all ovaries in which we counted oocytes (Fig. 3A). Cortical alveolar oocytes were present only in ovaries from fish longer than about 400 mm and older than 2 years and were common only in fish longer than about 475 mm and older than 4 years (Fig. 3B). Vitellogenic oocytes were found only in fish longer than 450 mm and were common only in fish longer than 550 mm and older than 5 years (Fig. 3C). PAS-positive melanomacrophage centers were common only in females longer than about 550 mm and older than 5 years (Fig. 3D): the same length and age as those for females that contained vitellogenic oocytes.

Seasonality of gonad development

Bonefish gonadal activity was seasonal. Vitellogenic oocytes were present in greatest numbers during November–May, and their numbers declined during May–June (Fig. 4). There were no vitellogenic oocytes in any ovaries from females collected during August–September of any of the three summers during which we sampled. Cortical alveolar oocytes were present during all months but were least abundant during July–October. Primary growth stage oocytes were present in all females examined and made up at least 20% of the total number of oocytes present. PAS-positive melanomacrophage centers were most abundant in the gonads of spent and regressed males and females and were most abundant in ovaries at the end of the spawning season in June–August (Fig. 5). They were least abundant in ovaries immediately before the initiation of spawning in November, when recrudescence was complete and most ovaries were ripening to spawn during winter–spring. We saw no evidence of recent or imminent spawning, such as postovulatory follicles or fully hydrated females. Only six ovaries contained atretic hydrated oocytes, and no single histological preparation contained more than a few hydrated oocytes.

Seasonal GSI patterns suggest that bonefish spawned during a prolonged period from November to June (Fig. 6). Median GSI's were greatest during November–May and were least during July–September. The decrease in female GSI's during July–September corresponded with the decrease in the number of vitellogenic oocytes present in ovaries and with the increased abundance of spent and regressed fish during late summer.

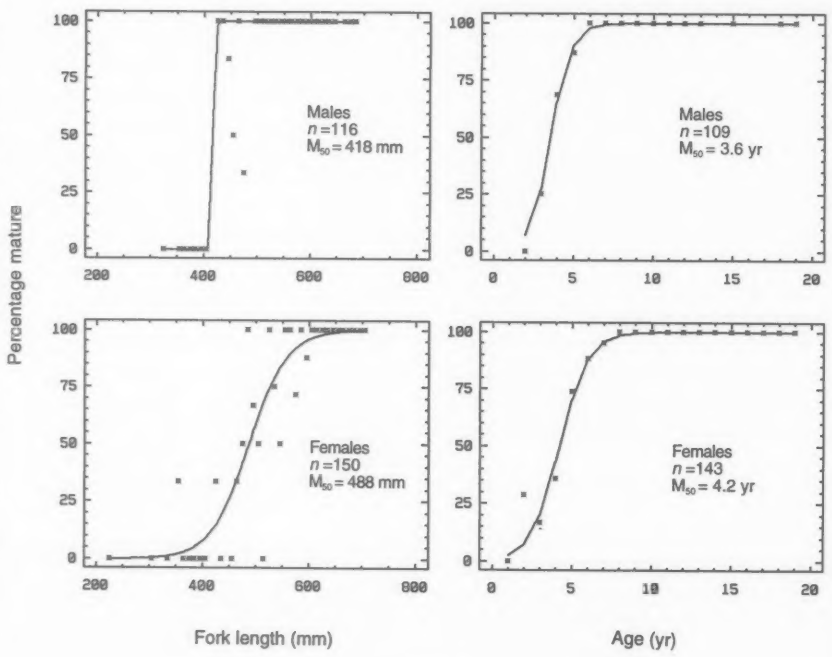

Figure 2

Length-maturity and age-maturity relations for male and female bonefish, *Albula vulpes*. Lengths plotted are the midpoints of 10-mm size classes. The solid line represents the predicted relation from the logistic regressions presented in Table 1. M_{50} is the length or age predicted by the logistic regression at which 50% of the bonefish were sexually mature.

Fecundity

Bonefish total fecundity estimates ranged from 0.4 to 1.7 million oocytes and had a significant positive relation to fish weight (Table 1; Fig. 7). Relative fecundity (the number of oocytes per gram fish weight) ranged from 159 to 385 oocytes/g (mean=259 oocytes/g, SD=47.1, n=33) for fish ranging in length from 485 to 702 mm. There was no significant relation between relative fecundity and fish length (n=33, r^2=0.014, P=0.514) or weight (n=33, r^2=0.043, P=0.247), but there was a significant positive relation between relative fecundity and age (n=32, r^2=0.248, P=0.003).

Oocyte development among areas within the ovary was homogeneous. We used a two-factor analysis of variance to compare oocyte densities with side (right or left) and position (anterior, middle, or posterior section of the ovary) as the effects. The number of late vitellogenic oocytes per gram of wet ovary weight was not significantly different between left and right ovaries (ANOVA, df=1, P=0.648) or among subsamples from anterior, middle, or posterior sections of the ovary (ANOVA, df=2, P=0.709). Furthermore, we found no significant interaction between side and position from which subsamples were taken (ANOVA, df=2, P=0.702). Weights of left and right ovaries from sexually mature females were not significantly different (paired t-test, n=112, t=0.480, P=0.632), but right testes from sexually mature males were significantly larger than left testes (mean difference=9.1 g, SD=18.62; paired t-test, n=98, t=4.826, P<0.001).

Discussion

Length and age at sexual maturity

The bonefish we examined reached sexual maturity at an older age and larger size than reported by Bruger (1974). He reported sexually mature females that were 1 year old and ranged from 221 to 352 mm FL (reported as 210 to 338 mm standard length). Bruger considered these small bonefish to be sexually mature on the basis of the presence of vitellogenic oocytes, one of the criteria that we used. He did not report how many of these small sexually mature fe-

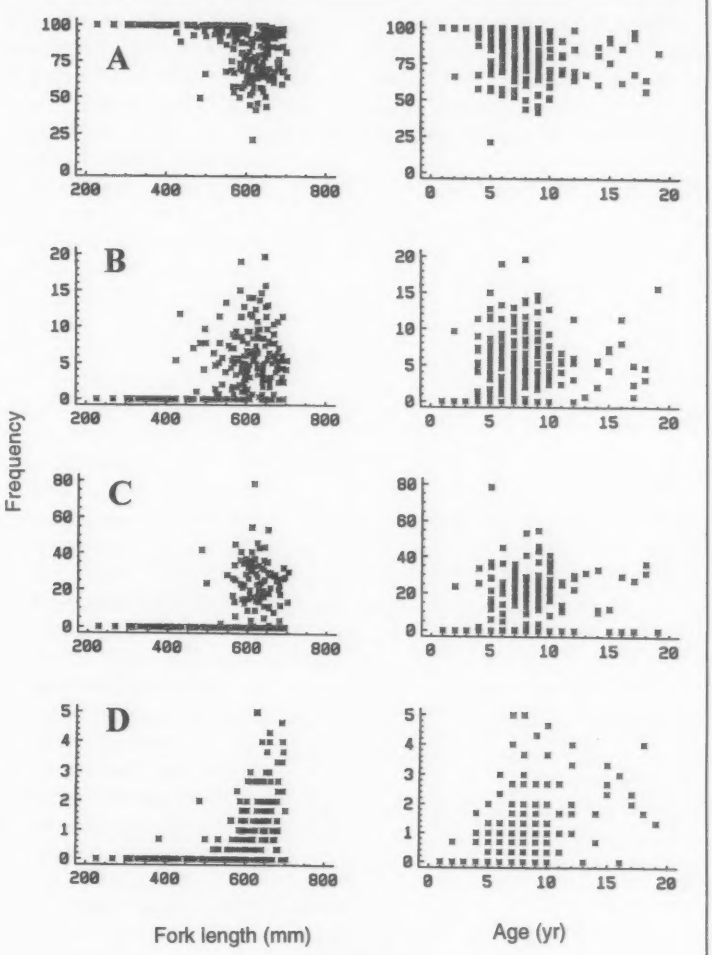

Figure 3

The percent frequency of occurrence of (**A**) primary growth oocytes, (**B**) cortical alveolar oocytes, (**C**) vitellogenic oocytes, and (**D**) PAS-positive melanomacrophage centers in bonefish, *Albula vulpes*, ovaries as a function of fork length (mm; n=248) and age (years; n=241).

males were captured or the typical length and age at sexual maturity. Some of the sexually mature females he collected were smaller than the 351-mm sexually mature female that we considered an outlier and excluded from our analysis. Furthermore, the lengths of Bruger's fish were substantially shorter than our estimated length at 50% maturity for females (488 mm). We also did not find any 1-year-old bonefish that were sexually mature; the youngest sexually mature fish we examined was 2 years old. All of the small mature females reported by Bruger were caught in deeper water (9.1–12.2 m) than that surveyed for bonefish in our sample; most of our fish were caught in water less than 2 m deep. Both Bruger (1974) and Crabtree et al. (1996) considered the possible existence of a cryptic bonefish species in waters off the Florida Keys as a potential explanation for the presence of exceptionally small and sexually mature bonefish, but additional study is needed to resolve this question.

Little is known regarding bonefish maturation in other areas. Pfeiler et al. (1988) reported 12 *Albula* sp., ranging in length from 205 to 264 mm (SL) from the Gulf of California, that had ripe or ripening gonads; this finding suggests a smaller length at sexual maturity there than we found in the Keys. The *Albula*

species in the Gulf of California is distinct from the Caribbean *A. vulpes* (Pfeiler, 1996).

Most of the bonefish in our sample that were caught with hook-and-line gear were 500–700 mm long (80%) and 3–10 years old (86%; Crabtree et al., 1996). If the length and age composition of our hook-and-line sample reflects that of the fishery, as suggested by Crabtree et al. (1996), then most of the fish caught in the fishery are longer than our estimate of length at 50% maturity and older than our estimate of age at 50% maturity. The current 390-mm-FL (457-mm-TL) legal minimum fish length imposed upon the fishery is less than our estimate of the length at 50% maturity for males (418 mm FL) and females (488 mm FL). Crabtree et al. (1996) found little evidence of fishing mortality in the Florida Keys because most bonefish caught by recreational anglers are released; thus, the population would probably be insensitive to changes in the legal minimum fish length.

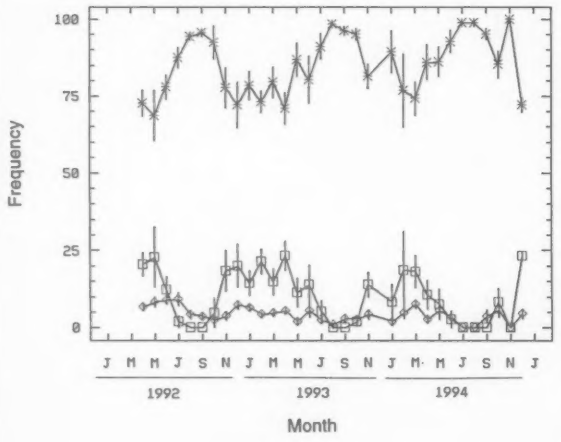

Figure 4

Monthly mean percent frequency of occurrence and standard errors of oocyte stages in bonefish, *Albula vulpes*, ovaries (n=250) for 1992–94. * = primary growth stage oocytes, ◇ = cortical alveolar oocytes, and □ = vitellogenic oocytes.

The presence of PAS-positive melanomacrophage centers was related to gonadal activity. They appeared to be associated with the resorption of postovulatory follicles and atretic vitellogenic oocytes, as has been suggested by Ravaglia and Maggese (1995). The presence of vitellogenic oocytes is an unambiguous indication of sexual maturity, and the length and age at which vitellogenic oocytes first developed in bonefish corresponded to the length and age at which PAS-positive melanomacrophage centers first appeared (Fig. 3). Melanomacrophage centers were most abundant at the end of the spawning season, when spent fish were most common, and decreased in abundance during the postreproductive period (July–November), when recrudescence occurred (Fig. 5). Ravaglia and Maggese (1995) found that the abundance of melanomacrophage centers was also seasonal in *Synbranchus marmoratus* and peaked during the postreproductive season.

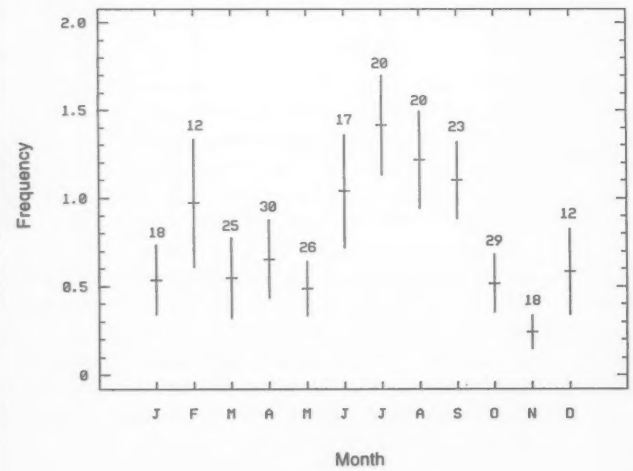

Figure 5

Monthly mean percent frequency of occurrence and standard errors of PAS-positive melanomacrophage centers in bonefish, *Albula vulpes*, ovaries. The numbers above the error bars are the numbers of ovaries examined.

Seasonality of gonad development

Bonefish gonadal activity in the Florida Keys was seasonal, and development occurred over about eight months, from November to June. Bonefish were reproductively inactive for only a few months during summer. This period of inactivity roughly corresponds with the period of maximum water temperatures in the Keys (Crabtree et al., 1996). Bruger (1974) reported no evidence of a seasonal pattern of gonadal development for bonefish off the Florida Keys, but his small sample size during July–September (n=7 females, n=4 males) may have obscured seasonal patterns. Alexander (1961)

found that the abundance of premetamorphic bonefish larvae in the West Indies was seasonal and that most larvae (96% of a total collection of 417 premetamorphic larvae) were caught during November–April. She concluded, from the capture of 17 premetamorphic larvae during August, that bonefish spawn throughout the year in the West Indies, but she suggested that bonefish spawn at reduced levels during the hot summer months. The 17 larvae that she reported were captured in August and were caught far south of the Florida Keys—at 13°11'N latitude, where spawning may follow a different seasonal pattern than it does in the Keys.

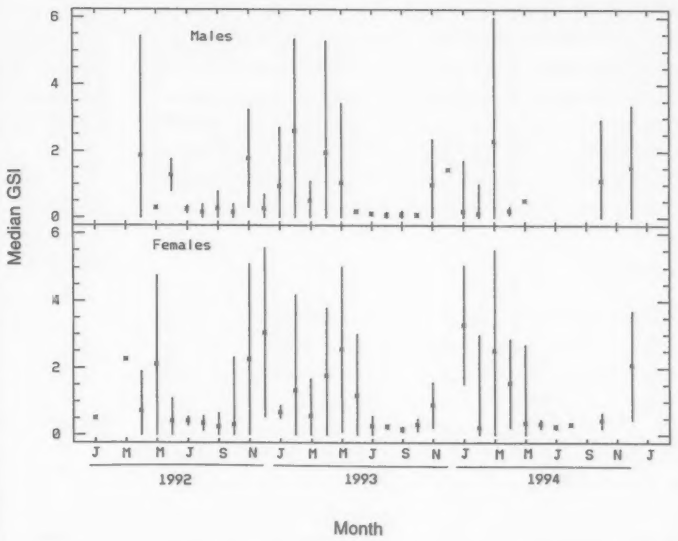

Figure 6

Monthly median gonadosomatic indices (GSI) and interquartile ranges for sexually mature male and female bonefish, *Albula vulpes*.

Studies from other areas also suggest seasonal reproduction in bonefish. Recruitment studies off the Bahama Islands suggest that spawning there has a seasonal pattern that is similar to that of spawning in the Florida Keys. Mojica et al. (1995) backcalculated hatching dates from the analysis of otolith microstructure of field-collected larvae and found that bonefish spawned continuously from mid-October through early January. Recruitment patterns suggest that spawning probably continues until spring, because Mojica et al. (1995) observed recruitment pulses of bonefish larvae as late as June—presumably resulting from April and May spawning. Bonefish spawning in the Gulf of California also appears to be seasonal. Pfeiler et al. (1988) examined gonads from 33 bonefish ranging from 202 to 279 mm (SL) and suggested that *Albula* sp. in the Gulf of California spawn during late spring and early summer. Metamorphic leptocephali are abundant in coastal regions and hypersaline lagoons throughout the Gulf of California during winter and spring (Pfeiler, 1984; Pfeiler et al., 1988). Pfeiler et al. (1988) suggested that the premetamorphic larval phase lasts 6 or 7 months.

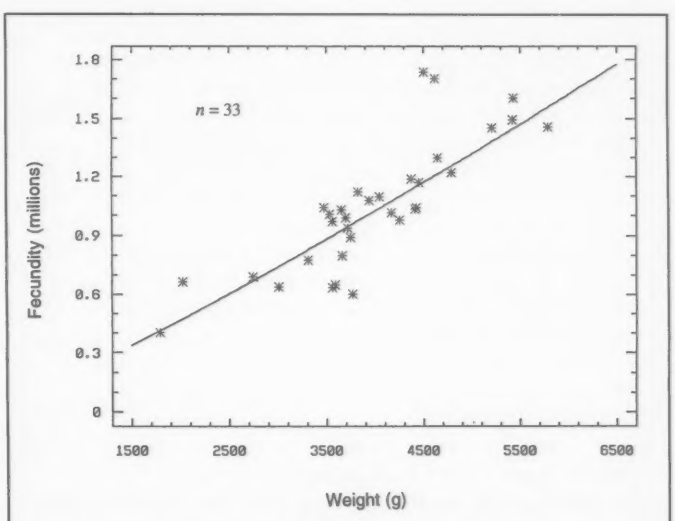

Figure 7

The fecundity-weight (g) relation for bonefish, *Albula vulpes*. The fecundity-weight regression equation is presented in Table 1.

The location of bonefish spawning grounds remains unknown, but the absence of females with postovulatory follicles or many hydrated oocytes in our samples suggests that bonefish do not spawn in the shallow nearshore areas where the fishery exists. It is possible that bonefish had spawned in our sampling area but did not feed before or after spawning and so were not available for capture with hook-and-

line gear until after they had completed the resorption of recognizable postovulatory follicles. This possibility seems unlikely because most collections of premetamorphic bonefish larvae are from offshore waters (Alexander, 1961); thus spawning bonefish probably move out of the shallow waters (<2 m) where fishing usually occurs. Alexander (1961) suggested that bonefish either spawn offshore or in areas where currents are likely to carry the eggs offshore.

Fecundity

We did not examine any bonefish ovaries containing oocytes in the final stages of oocyte maturation or showing definitive evidence of recent spawning, such as postovulatory follicles. Consequently, we do not know if bonefish are isochronal or multiple-batch spawners, and we could not estimate batch fecundity. If annual fecundity in bonefish is indeterminate (Hunter et al., 1992), our estimate of total fecundity may not accurately represent total annual egg production. The bonefish spawning season is prolonged, and the potential exists for additional vitellogenic oocytes to mature from the standing stock of unyolked oocytes during the spawning season. Some ovaries contained vitellogenic oocytes, widespread atresia, and were loosely organized and highly vascularized. These females may have spawned earlier in the season and were developing an additional batch of oocytes that would have been spawned later in the season. It is unclear whether these oocytes were recruited from the standing stock of unyolked oocytes after previous spawning or if they were vitellogenic oocytes already present in the ovary that did not ovulate during previous spawning. Another bias of our fecundity estimates is that we could not correct them for atretic losses of vitellogenic oocytes during the spawning season; these losses could have caused us to overestimate egg production.

Acknowledgments

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Seasonal movement of offshore American lobster, *Homarus americanus*, tagged along the eastern shore of Cape Cod, Massachusetts

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Abstract.—A total of 1,237 tagged American lobsters, *Homarus americanus*, with a carapace length (CL) range of 48 to 198 mm (mean CL of 104 mm) were liberated at three release stations off the eastern shore of Cape Cod, MA, between 1969 and 1971. By 1973, 332 (26.8%) of the tags were returned. Mean time at large was 112.5 days (range 0–897 d).

One hundred and thirty (39.2%) of the recaptured lobsters moved less than 10 km from their points of release. One hundred and fifty-one (45.5%) were recaptured within 10 to 40 km from their points of release; 51 (15.4%) at 40 km or more.

Recapture depths and distances traveled were significantly greater in colder months. The distribution of these recaptures with time, depth, and location indicates seasonal movement to and from the edge of the continental shelf between fall and spring.

The apparent reshoaling of these inshore-tagged lobsters to the eastern shore of Massachusetts in successive summers and the greater movement shown by females with ripe eggs at tagging, versus the movement of sublegal and nonovigerous female classes, suggest that the migration of this group of offshore lobsters is stimulated by seasonal changes in environmental cues in relation to hatching or reproductive needs (or both). Their relation to the Georges Bank–Southern Offshore stock unit, reproductive potential, and extensive seasonal movement into the southern and western Gulf of Maine, represent important considerations for resource managers and emphasize the need for further research on rate of stock interchange.

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The traditional American lobster, *Homarus americanus*, fishery consists of a small-boat fleet that fishes with traps within a few miles of shore in depths up to 20 fathoms. This inshore fishery is centered in the northern Gulf of Maine and produces annually approximately 47% of the U.S. pounds landed. Massachusetts, the next largest producer, contributes about 28% of U.S. landings. Exploitation of inshore stocks is intensive. In the coastal waters of Maine, over 85% of the commercial inshore catch consists of new recruits (Krouse et al.[1]). In Massachusetts coastal waters, approximately 90% of the inshore catch falls into this category (Estrella and Armstrong[2]).

Prior to 1948, small numbers of lobsters were caught incidentally by trawls in groundfish operations. These incidental catches accounted for less than 1% of U.S. landings. About 1945, trawlers began to fish specifically for lobsters, principally in deep water in the offshore region south of the Gulf between southeast Georges Bank and Hudson Canyon. This fishery was developed moderately and by 1968 offshore lobsters accounted for 16.9% of all U.S. lobster landings (Skud and Perkins, 1969). The introduction of deep-water trap fishing in the late 1960's rapidly accelerated development of the offshore fishery. Vessels that fish with traps have largely replaced the original trawl fleet, and the number of vessels in the offshore lobster fishery have increased greatly. Substantial numbers of vessels in the 40' to 60' class, as well as larger vessels, were built or converted specifically for offshore trap fishing. Offshore landings averaged 24% of U.S. landings (3,400 metric tons [t]) between 1970 and 1974 but declined to a 1978–83 average of 17% or 2,500 t per year (NEFC, 1983). Despite short-term increases (5,000 t in 1990), offshore landings have not accounted for more than 18% of total U.S. lobster landings since the mid 1970's (NEFSC, 1994).

[1] Krouse, J. S., K. H. Kelly, G. E. Nutting, D. B. Parkhurst Jr., G. A. Robinson, and B. C. Scully. 1994. Maine Dep. Marine Resources lobster stock assessment project 3-IJ-61-2. Maine Dep. Mar. Resources, Marine Resources Laboratory, P.O. Box 8, West Boothbay Harbor, ME 04575. Annual Rep., 53 p.

[2] Estrella, B. T., and M. P. Armstrong. 1995. Massachusetts coastal commercial lobster trap sampling program, May–November, 1994. Mass. Div. Mar. Fish., 20 p.

The continued development of the offshore fishery has subjected American lobster in all remaining segments of its range to intensive exploitation. Thus, stock identification and determination of any interrelations between stocks of lobsters is of increasing importance to management of the lobster fishery (Pezzack[3]). Earlier studies indicated that lobsters were relatively nonmigratory. Numerous tagging experiments conducted primarily in more northern inshore areas showed that most lobsters usually remain within a radius of 3–5 km (Templeman, 1935, 1940; Wilder and Murray, 1958; Wilder, 1963; Cooper, 1970; Cooper et al., 1975; Krouse, 1980; Stasko, 1980; Campbell, 1982; Lawton et al., 1984). Accordingly, management practices were based largely on the concept of discrete local stocks. Findings of extensive lobster movement in tagging experiments conducted in offshore areas (Saila and Flowers, 1968; Cooper and Uzmann, 1971, 1980; Uzmann et al., 1977; Fogarty et al., 1980; Campbell et al,. 1984) and in more southern inshore areas (Morrissey, 1971; Briggs and Muschacke, 1984) show significant movement of large, sexually mature lobsters which become intermixed with the inshore resource. Thus, the concept of discrete inshore stocks, characterized by a particular size range or maturity status (Campbell and Stasko, 1986; Campbell, 1989) becomes speculative.

Intermingling of offshore lobsters with inshore stocks off southern New England is shown from recaptures of offshore-tagged lobsters in inshore areas. Cooper and Uzmann (1971, 1980) and Campbell (1986) hypothesized that seasonal depth-related movements are important to the biology of the species in providing optimal temperatures for mating, molting, egg extrusion, and egg development.

In an effort to obtain information that would augment offshore tagging studies off the Massachusetts coast, we undertook a three-year tagging experiment beginning in 1969 in the inshore waters of Cape Cod where previous work (Morrissey, 1971) showed the existence of a seasonal population of large, highly mobile lobsters. Additional lobster tagging in this area in 1984–85 also confirmed highly migratory behavior.[4]

Estrella and McKiernan (1989) described the extensive size range of this segment of the lobster resource, which is only seasonally available east of Cape Cod, as characteristic of an offshore migrant group. This area exhibits the smallest percentage of sublegal-size lobsters in commercial trap catches of any other Massachusetts coastal region (10% compared with 89% in waters off Boston, MA, in 1995[5]). Catch per trap haul of sublegal-size lobster off outer Cape Cod was four to eight times lower than that for other Massachusetts coastal regions sampled in 1994 (Estrella and Armstrong[2]). Outer Cape Cod habitat is not "classic" lobster habitat conducive to supporting a resident (burrowed) resource; it is characterized by expansive sandy bottom and is dynamic owing to its exposure to strong easterly winds. Some local lobster production apparently occurs in the Nauset Marsh area of outer Cape Cod where limited numbers of early benthic-phase lobster have been found (Able et al., 1988). However, it is the larger, more common, offshore migrant lobster which supports the commercial fishermen in this area and which shapes the style of fishing deployed there. Long strings of traps are set parallel to the shoreline to intercept incoming migrations each season. In late spring, traps are initially set by day-boat lobstermen approximately thirteen miles from shore. This gear is gradually fished shoalward as migrations proceed closer to land in warmer months, until declining autumn temperatures reverse the trend.

It is informative that the intense outer Cape Cod lobster fishery has not been successful in reducing the size structure of this resource as definitively as in other inshore Massachusetts regions. A greater number of size-age groups are represented in outer Cape Cod catches. In most other inshore areas, lobsters exhibit minimal migration and are exposed to fishing pressure throughout the year. New recruits (lobsters which, upon molting, become legal size) may represent as much as 95% of the legal catch, compared with only 55% in the outer Cape Cod area. The seasonal occurrence of these offshore lobsters in the outer Cape Cod area thus limits their exposure to intense fishing pressure.

The size structure of this portion of the resource is similar to that from southern Georges Bank. Accordingly, the Sixteenth Northeast Regional Stock Assessment Workshop (16th SAW) of NMFS assigned this migratory group of lobsters to the Georges Bank-Southern Offshore stock unit (NEFSC, 1993).

Estrella and McKiernan (1989) discussed the geography as a potential factor in concentrating migrants. The outer Cape Cod area is adjacent to steeply sloping gradients which lead to a much greater depth range than that found in most other inshore regions.

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[3] Pezzack, D. S. 1987. Lobster (*Homarus americanus*) stock-structure in the Gulf of Maine. Int. Counc. Explor. Sea. Shellfish Comm. Council Meeting 1987/K:17, 18 p.

[4] Estrella, B. T. 1997. American lobster tagging studies conducted in Massachusetts coastal waters. MA Div. Mar. Fish. In prep.

[5] Estrella, B. T. 1997. Massachusetts Division of Marine Fisheries, 50 A Portside Drive, Pocasset, MA 02559. Unpubl. data.

Materials and methods

Tagged lobsters were released at three specific locations along the eastern shore of Cape Cod (Fig. 1). Lobsters used in tagging were collected in the immediate vicinity of each release station and released within a day of capture. At station 1 (Provincetown), tagged lobsters were liberated in the periods 21–25 July 1969; 6–9 July 1970; and 23–25 June 1971. Lobsters used in the tagging at station 1 were collected by SCUBA teams that attempted to capture all lobsters observed on each dive. At station 2 (Truro) and station 3 (Eastham), tagged lobsters were liberated over the period 20 July to 18 August 1970. Lobsters used at these two stations were collected in the traps of a local commercial fisherman.

Sphyrion anchor tags were used. These consisted of a coded polyvinyl-chloride-tubing pennant connected by a monofilament thread to a stainless steel anchor. The anchor was inserted in the lobsters with a hypodermic needle through the membrane connecting the carapace and first abdominal segment and implanted in dorsal extensor musculature below the carapace hypodermis as described in Cooper (1970). The implanted tag can endure successive molts.

A reward of $1 was paid for each tag, as well as the market value of each tagged lobster returned with information on the date and location of recapture. During 1971, the reward was increased to $5 for each tagged lobster submitted for examination, and the fisherman was permitted to retain possession of the lobster.

Distance traveled by recaptured lobsters was determined as the shortest distance, avoiding landmass, from point of release to point of recapture. Direction of travel was computed to the nearest 0.1 degree true north.

Results

A total of 1,237 tagged lobsters with carapace length (CL) range of 48 to 198 mm (mean CL of 104 mm),

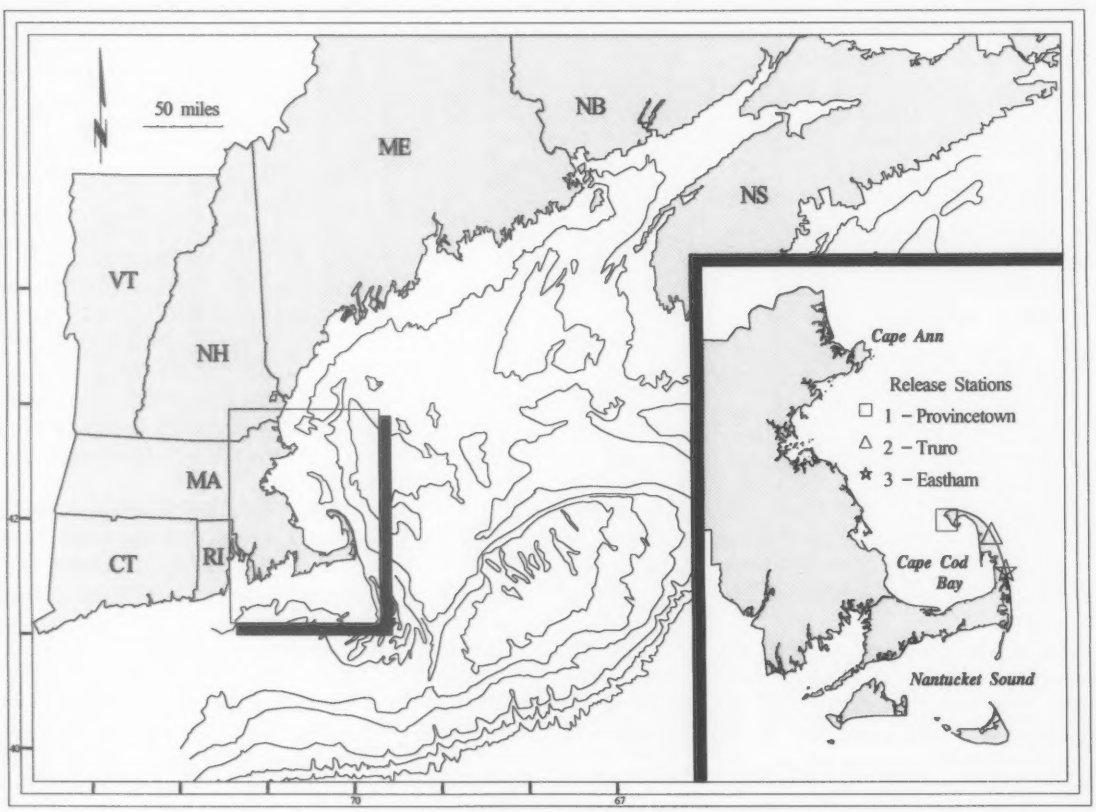

Figure 1

Map of northeast coast of the United States and Canada showing eastern Cape Cod, Massachusetts, release stations for tagged American lobster, *Homarus americanus*.

were liberated at three release stations (Table 1) between 21 July 1969 and 25 June 1971. By 22 December 1972, 332 or 26.8% of the tags were returned (Fig. 2). Mean distance from point of release to point of recapture was 22.6 km (median=140.4 km), mean time at large 112.5 days (median = 448.5 d).

One hundred thirty (39.2%) of the recaptured lobsters moved less than 10 km from their points of release (Table 2). One hundred fifty-one (45.5%) were recaptured within 10 to 40 km, and 51 (15.4%) were recaptured at 40 km or more from point of release. Four lobsters moved farther than 100 km, one of the four as far as 281 km.

The distances traveled by lobsters grouped in classes based on size, sex, and the presence or absence of ripe and immature external eggs at tagging are shown in Table 3A. Legal-size females without eggs moved the shortest distance of all groups. Ripe ovigerous lobster moved farthest (average 30.3 km), followed by females with immature eggs (average 23.8 km). Analysis of variance of log-transformed data indicated there were significant differences among groups ($P<0.01$). Several multiple-range test procedures, Tukey-HSD test, Student-Newman-Keuls (SNK), and Duncan, were run on log-transformed data. A common result among the tests was

Table 1

Tagged lobsters liberated and recaptured from three release stations at Cape Cod, Massachusetts.

Release station	Number tagged	Carapace length (mm)			Tags returned		Movement		
		Mean	Range	SD	Number	Percent recovery	Mean distance traveled (km)	Mean time at large (days)	Velocity (km/day)
1 Provincetown									
Male	190	84	48–198	25.2	54	28.4	22.4	220.4	0.49
Female	683	113	49–189	21.2	200	29.3	21.4	83.6	0.65
2 Truro									
Male	39	75	67–80	3.8	8	20.5	25.3	228.9	0.37
Female	105	106	67–149	21.0	21	20.0	21.7	104.7	1.50
3 Eastham									
Male	75	76	69–90	3.8	14	18.7	22.6	198.1	0.49
Female	145	96	66–146	21.6	35	24.1	29.6	55.1	1.18
Total or weighted mean	1237	102	48–198	24.9	332	26.8	22.6	112.5	0.72

Table 2

Distance traveled by lobsters liberated from three release stations at Cape Cod, Massachusetts.

Distance traveled (km)	Provincetown		Truro		Eastham		Total	
	Number of tags returned	Percent of total	Number of tags returned	Percent of total	Number of tags returned	Percent of total	Number of tags returned	Percent of total
Less than 10	100	39.4	2	6.9	28	57.1	130	39.2
10–19	49	19.3	15	51.7	1	2.0	65	19.6
20–29	26	10.2	2	6.9	2	4.1	30	9.0
30–39	46	18.1	8	27.7	2	4.1	56	16.9
40–49	20	7.8	1	3.4	5	10.3	26	7.8
50 or more	13	5.2	1	3.4	11	22.4	25	7.5
Total	254	100.0	29	100.0	49	100.0	332	100.0

that the distance traveled by legal-size nonovigerous females was significantly shorter than that of all other groups except sublegal females.

Only fourteen of the returned lobsters molted while at large. These were distributed among most of the lobster classes. Sample size was insufficient to assess effects of molting on movement.

The relatively long mean distances traveled by sublegal male and female lobster groups (22.5 km and 17.0 km, respectively) were likely due to their number of days at large being, on average, considerably greater than the number of days at large for other lobster groups (Table 3A). The mean time at large was less for legal-size females with and without external eggs than for legal-size males and sublegal males and females.

Lobster "velocities" greater than 3 km/day were not exhibited by individual sublegal males or sublegal females. However, these rates were calculated for the larger lobsters, including 6.6% of legal-size males, 1.1% of legal-size nonovigerous females, 7.6% of females with immature eggs, and 4.2% of females with ripe eggs. Females with immature and ripe eggs exhibited greatest mean velocities (1.55 and 0.95 km/day, respectively, Table 3A).

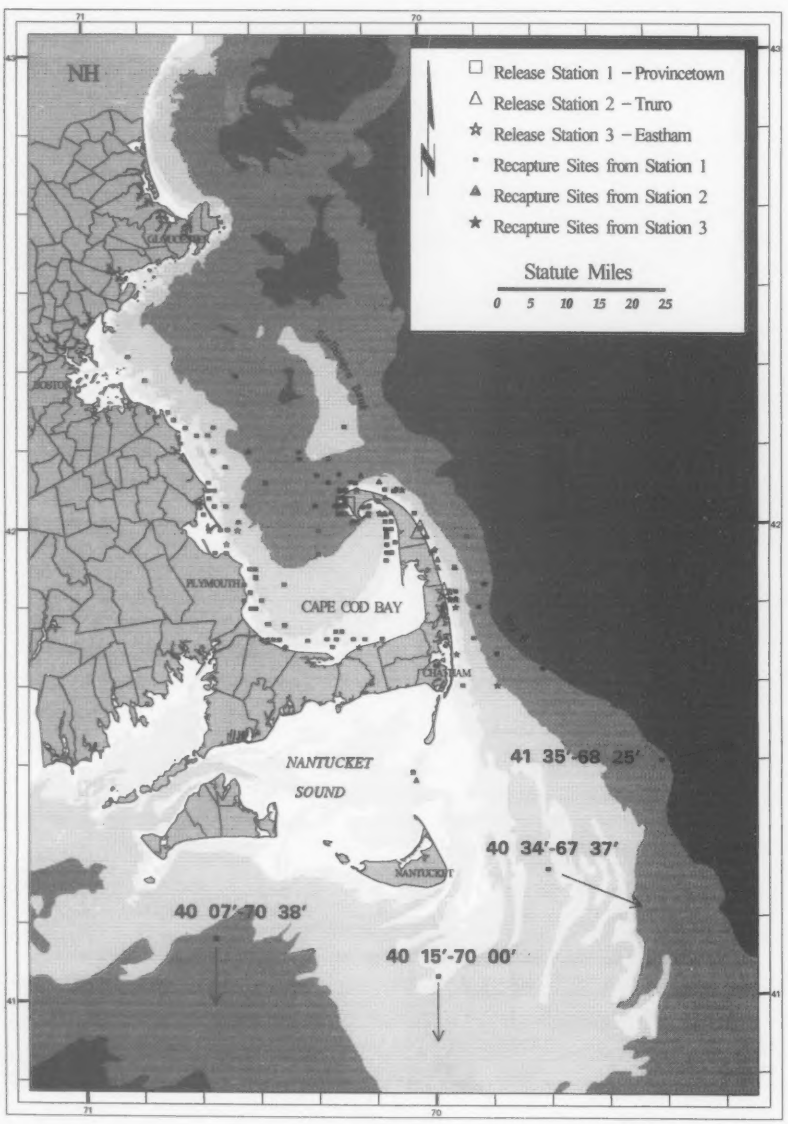

Figure 2

Tagged American lobster, *Homarus americanus*, release stations and return locations off the coast of Massachusetts. Some inshore recapture sites represent multiple recaptures.

Because variability in days at large among classes of lobster could affect comparisons of distance traveled, standardization was warranted. An additional data analysis was conducted which was limited to lobsters at large < 200 days (Table 3B). This eliminated potential misleading recapture locations that could occur after circuitous (homing) movement patterns, i.e. those from lobsters which, after tagging, may move offshore and return inshore in the following year, and subsequent years. Because lobsters were tagged and released in the months of June through August, a 200-day limit was considered reasonable to avoid spring recaptures in our data treatments.

Analysis of these "standardized" data reaffirmed that legal-size females with ripe external eggs exhibited the greatest mean distance traveled, 25.6 km, followed by females with immature external eggs, 24.2 km. Legal-size males ranked third, with a mean of 16 km; nonovigerous females and sublegal females and males averaged 12.2 km, 13.1 km, and 14.2 km, respectively. Analysis of variance of log-transformed distance data indicated that there were signifi-

Table 3A

Tag returns from various classes of lobsters liberated at Cape Cod, Massachusetts, for all days at large.

Lobster class	Number liberated	Number recovered	Percent tag recovery	Mean carapace length (mm)	Mean distance traveled (km)	Mean time at large (days)	Velocity (km/day)
Male sublegal-size[1]	213	46	21.6	72	22.5	308.0	0.31
Female sublegal-size without external eggs	128	20	15.6	75	17.0	153.0	0.37
Male legal-size	91	30	33.0	106	23.1	79.0	0.74
Female legal-size without external eggs	295	91	30.8	106	13.4	77.0	0.46
Female with immature external eggs	130	26	20.0	112	23.8	34.2	1.55
Female with ripe external eggs	380	119	31.3	118	30.3	83.0	0.95
Total or weighted mean	1,237	332	26.8	104	22.6	112.5	0.72

[1] Lobsters less than 81 mm carapace length. During this study, the minimum legal carapace length in Massachusetts was 3 and 3/16 inches (80.96 mm).

Table 3B

Tag returns from various classes of lobsters liberated at Cape Cod, Massachusetts, which were at large <200 days.

Lobster class	Number liberated	Number recovered	Percent tag recovery	Mean carapace length (mm)	Mean distance traveled (km)	Mean time at large (days)	Velocity (km/day)
Male sublegal-size[1]	213	21	9.9	75	14.2	34.1	0.59
Female sublegal-size without external eggs	128	14	10.9	75	13.1	47.2	0.51
Male legal-size	91	26	28.6	104	16.0	38.1	0.82
Female legal-size without external eggs	295	81	27.5	106	12.2	35.0	0.51
Female with immature external eggs	130	25	19.2	112	24.2	22.8	1.61
Female with ripe external eggs	380	107	28.2	117	25.6	36.1	1.04
Total or weighted mean	1,237	274	22.2	107	19.1	35.2	0.85

[1] Lobsters less than 81 mm carapace length. During this study the minimum legal carapace length in Massachusetts was 3 and 3/16 inches (80.96 mm).

cant differences among groups ($P<0.01$). Tukey-HSD, SNK, and Duncan test results were similar to results from tests on all data. They indicated that distances traveled by sublegal and legal nonovigerous lobster groups were significantly less than those of egg-bearing female groups ($P=0.05$). The trend in mean velocity and proportion of lobsters traveling greater than 3 km/day was similar to that calculated for each lobster class from all data. Maximum velocities, calculated by lobster class, were 2.98 km/day for a 79-mm-CL sublegal male, 2.36 km/day for sublegal females (80 mm CL), 5.19 km/day for legal size males (106 mm CL), 4.15 km/day for legal-size nonovigerous females (101 mm CL), 7 km/day for le-

gal-size females with immature eggs (103 mm CL), and 5.8 km/day for females with mature eggs (118 mm CL).

Time at large for recaptured lobsters ranged from 0 to 897 days (Table 4). Approximately 78% of recaptured lobsters were at large less than 60 days. The number of tags returned and the percentage recovery of tagged lobsters at large decreased sharply in the fourth month after release (October) coincident with the start of the fall season. Only three tags were returned from lobsters recaptured in the colder months from December through April (all years combined). All three tagged lobsters were recovered in deep water. Two were recaptured on 7 and 22 March 1972, at depths of 54 m and 59 m, respectively, off Provincetown, MA, and the third was recaptured offshore on 22 December 1972, at a depth of 95 m, NE of Veatch Canyon.

Depth of recapture data were log-transformed and analyzed with equality of means test and found to be related to season (Welch: F=4.41, P=0.0411; Brown-Forsythe: F=4.41, P=0.0411). Depth of recapture for the combined months of June through September (63 m) was significantly different from October through May (93 m).

Distance traveled was also significantly different by season (t=–3.50, P=0.001). Distance from release site was greatest during the October–May period of recapture (41.3 km), in comparison with June–September period of recapture (22.5 km).

Distance of travel northeastward of the Cape Cod landmass was apparently limited compared with distance of travel in other directions; recapture points tend to be distributed in a northwest-southeast plane (Fig. 2).

To test inshore versus offshore migrational tendencies, tag-return locations were grouped with consideration for the curvature of the "arm" of the Cape. Exclusive of lobster recaptured within Cape Cod Bay, recapture points east to south of all landmass between 90° and 225° true north (for lobsters liberated at stations 2 and 3), and 1° and 225° (for lobsters

Table 4

Tags returned by month of recapture and days at large for tagged lobsters liberated at Cape Cod, Massachusetts (recapture years combined).

Recapture month	Mean days at large	Number of returns	Tags returned		Mean distance (km)	Mean depth (meters)
			Percent of total	Cumulative percent		
1st season						
June	4.0	2	0.6		3.2	70.0
July	10.1	70	21.1	21.7	10.1	62.5
August	33.2	112	33.7	55.4	20.0	53.0
September	50.6	76	22.9	78.3	26.2	78.5
October	89.9	9	2.7	81.0	21.1	81.2
November	111.0	5	1.5	82.5	19.5	128.2
2nd season						
March	258.0	1	0.3	82.8	4.6	176.0
May	302.1	10	3.0	85.8	69.7	91.3
June	338.8	9	2.7	88.5	22.5	54.3
July	359.8	5	1.5	90.0	37.0	33.8
August	381.6	8	2.4	92.4	33.1	51.9
September	434.5	2	0.6	93.0	7.6	92.5
October	449.8	4	1.2	94.2	39.8	48.3
November	486.0	1	0.3	94.5	33.8	50.0
3rd season						
March	582.0	1	0.3	94.8	38.5	192.0
May	670.0	2	0.6	95.4	19.9	30.5
June	703.5	2	0.6	96.0	35.7	60.5
July	726.3	6	1.8	97.8	23.2	81.5
August	742.5	2	0.6	98.4	33.9	93.0
October	807.5	2	0.6	99.0	30.2	70.0
November	843.5	2	0.6	99.6	11.5	61.0
December	897.0	1	0.3	99.9	223.5	312.0

liberated at station 1) were grouped as southeast (offshore) in direction. All other recapture points, including those within Cape Cod Bay, were grouped as northwest (inshore) in direction. Inshore versus offshore travel was tested with chi-square by month of recapture for lobsters at large up to one year and which traveled more than 3.7 km. Direction of travel was biased significantly toward inshore during warmer months (Table 5).

Discussion

Our findings of greater directed movement toward the north and west during summer months is in agreement with the summer movement along the eastern shore of Cape Cod and into Cape Cod Bay as reported by Morrissey (1971). The Cape Cod landmass is interjacent to inshore grounds to the north and west and offshore grounds along the edge of the continental shelf to the south and east. The overall northwest-southeast distribution of recapture points suggests an interchange of inshore and offshore stocks in the Cape Cod area. Cooper and Uzmann (1971) and Uzmann et al. (1977) found that lobsters tagged in offshore canyon areas moved into shoal water in late spring and early summer, returning to deep water in late fall and early winter. Lobsters migrating from that offshore area to the inshore area of eastern Massachusetts would pass along the eastern shore of Cape Cod. The northwest-southeast pattern of recapture locations is consistent with movement to and from the Georges Bank and southern offshore canyon area which exhibits a similar population structure to the lobster group which is only seasonally available east of Cape Cod.

Table 5

Seasonal distribution of recapture points of lobsters at large up to one year and that had traveled more than 3.7 km.

	Direction of travel			
Recapture period	Number inshore	Number offshore	Total	χ^2
1st season of release				
July–August	106	43	149	$P<0.0001$
September–October	59	15	74	$P<0.0001$
1st winter of release				
November–May	11	5	16	$P=0.134$
2nd season of release				
June–August	8	4	12	$P=0.248$
Total	184	67	251	

Lawton et al. (1984) found minimal movement in an inshore tagging study of 4,761 sublegal lobster at nearby Rocky Point, Plymouth, Massachusetts during 1970–75. Only 19 lobsters (<1% of returns) were retrieved 16 or more km from their release points and all 19 were within state territorial waters. A study by Fair[6] on legal-size lobster in the same area several years later yielded similar results. Additional studies affirmed the nonmigratory nature of inshore lobsters (Templeman, 1935; Wilder, 1963; Cooper et al., 1975; Krouse, 1980; Stasko, 1980; Campbell, 1982). Ennis (1984) noted small-scale seasonal depth movements in relation to temperature with lobsters moving to shallow water in warmer months and deeper water in colder months. More extensive seasonal migrations were demonstrated by Campbell (1986) and Pezzack and Duggan (1986).

We conclude that lobsters tagged in the present experiment are onshore migrants from an offshore stock that seasonably becomes "superimposed" on the endemic inshore stock. Recapture depths were significantly greater in colder months than during summer. The movement of lobsters off southeastern Massachusetts is cyclical, with lobsters moving to deep water in late fall. Uzmann et al. (1977) found that lobsters returned to the continental shelf margin and slope in fall and winter. In our study only three lobsters were recaptured during December through April, consequently, we did not clearly establish if lobsters winter specifically on the edge of the shelf or in deep-water areas in general. However, four of our lobsters were recaptured in clearly offshore areas: at 40°07', 70° 38', 119 m depth, on 26 September 1970; at 40°34', 67°37', 110 m depth, on 9 May 1970; at 41°35', 68°25', 55 m depth, on 23 May 1971; and at 40°15', 70°00', 95 m depth, on 22 December 1972. The occurrences of these recaptures in time, depth, and location suggest seasonal movement to and from the edge of the continental shelf between fall and spring. With an average recovery rate of only 7.0% of lobsters tagged offshore by Cooper and Uzmann (1971), it is probable that substantial numbers of our inshore tagged lobsters wintered on the edge of the continental shelf.

Cooper and Uzmann (1971) concluded that offshore lobsters actively orient to optimum temperature according to season. Uzmann et al. (1977) provided further sup-

[6] Fair, J. J., Jr. 1977. Lobster investigations in management area 1: southern Gulf of Maine. NOAA, NMFS State-Federal Relationships Div., Mass. Lobster Rep. No. 8, 21 April 1975–20 Apr. 1977, 8 p. Appendix, 5 p.

port with their finding that through random or directed movements (or both), the offshore lobster population maintains itself within temperatures of 8°–14°C. Cooper and Uzmann (1971) hypothesized that seasonal shoalward migration to warmer water compensates for a lack of sufficiently high temperature during summer in the continental slope habitat to permit extrusion and hatching of eggs and subsequent molting and mating. They found that offshore lobsters that demonstrated the most extensive onshore migrations were predominately females and that the migration of offshore lobsters to inshore grounds is generally confined to areas south and west of Cape Cod (no recoveries were made north of Cape Cod in the Gulf of Maine proper).

While diving to collect lobsters for tagging at station 1, we observed lobsters always to be concentrated in a narrow stratum where a thermocline intercepted the steeply sloping surfaces of a sandy escarpment paralleling the beach in that area, about 1.8 km from shore. Morrissey[7] conducted semiweekly SCUBA surveys throughout the summer of 1966 at Provincetown, where station 1 is located, and found lobsters only in close proximity to the thermocline-sediment interface, which occurred at 24 m in late May and ranged between 9 and 19 m during June, July, and August. During a vertical transect along the bottom from the shoreline to a depth of 22 m, 14 July 1966, 15 of 16 lobsters observed were within a stratum (11 to 14 m) in which a bathythermograph cast showed a change of 12.8°C in water temperature. On the basis of observed activity of individual lobsters, he concluded that the lobsters were not concentrated by a thermal block but rather were attracted to the warmer epilimnion layer and used the reduced light intensity associated with the thermocline as cover. These observations support the conclusion of Cooper and Uzmann (1971) and Uzmann et al. (1977) that offshore lobsters orient to optimum temperature.

Our test results indicated that sublegal and legal-size females with no eggs moved significantly less than egg-bearing female groups. An explanation for why legal-size females without eggs move less than those with eggs may be that they tend to congregate in the warmer shoal water where egg extrusion occurs. During this study, three tagged females, which extruded eggs after tagging, moved only an average distance of 4.2 km before recovery. Although this sample size is small, the inference from it is supported by fishing activity in this area. Fishermen report concentrations of females with immature eggs in the shoals east of Cape Cod during August and September.

The fact that tagged inshore female lobsters with ripe external eggs moved greater distances than other classes of tagged lobsters may be a verification of the findings of Cooper and Uzmann (1971), that offshore lobsters demonstrating the most extensive inshore migrations are predominantly female. However, unlike Cooper and Uzmann (1971), our recoveries indicate that lobster migration occurs north of Cape Cod into at least the southwestern portion of the Gulf of Maine with one recovery made as far north as latitude 42°39'. Our subsequent tagging work in this study area (1984–85) yielded the return of a female after 362 days at large from even farther north, 43°33' (off Cape Elizabeth, Maine).[4]

None of our lobsters were recovered in the inshore grounds south and southwest of Cape Cod where most of the inshore recoveries were made by Cooper and Uzmann. The distribution of recapture points of the 58 lobsters recovered after their first season of release (Table 4) suggests that our inshore tagged lobsters returned to the shoal waters along eastern Massachusetts in successive summers.

The movement described by the findings of Cooper and Uzmann (1971) suggests that offshore lobsters migrate to secure more suitable hydrographic conditions. The areas involved, i.e. the edge of the continental shelf and shoaler waters extending into inshore grounds south and west of Cape Cod, are quite generalized. The apparent return of our inshore tagged lobsters to the eastern shore of Massachusetts in successive summers, and the greater movement shown by females with ripe eggs at tagging, suggest that the migration of offshore lobsters may be anastrophic or gametic (Heape, 1931; Wilkinson, 1952) in character, i.e. nonrandom, stimulated by metabolic needs or reproductive cues. Campbell (1986) provided calculations that suggest ovigerous lobster need to make seasonal deep-shallow water migrations to obtain sufficient heat units for egg development within any 9–12 month period. Using a threshold temperature of 3.4°C, he determined that shallow water had more degree days than deeper water in summer months and that the reverse was true in winter months.

Although significant American lobster migrations have been reported, Saila and Flowers (1968) provided the first reference in the literature to long-distance homing by this species. They found a pronounced directional tendency toward the original area of capture in the movements of berried female lobsters transplanted from Veatch Canyon on the edge of the continental shelf to Narragansestt Bay,

[7] Morrissey, T. D. 1970. Observations on behavior of the American lobster, *Homarus americanus*, at Provincetown, Massachusetts during the summer of 1966. MA Div. Mar. Fish., 50 A Portside Drive, Pocasset, MA 02559.

Rhode Island. They concluded that the lobsters tended to remain in shoal waters in suitable spawning habitat until they had shed their eggs or had molted, or both. Cooper and Uzmann (1971) referred to seasonal movement to and from generalized areas: the edge of the continental shelf and the shoaler waters of southern New England. Campbell (1986) and Pezzack and Duggan (1986), however, provided evidence from Canadian waters that lobsters undertake regular migrations between widely spaced and well-defined areas.

In contrast, the European lobster, *Homarus gammarus*, although biologically similar to *H. americanus*, displays minimal migratory behavior (Bannister and Addison, 1995). Tagging studies have shown that both juveniles and adults exhibit "strong site loyalty." The distribution of the *H. gammarus* resource and fishery is primarily coastal; "offshore" distribution occurs only 20 km from shore. The lack of substantial long-distance movement may be due to the more moderate water temperature off the British Isles (compared with the NW Atlantic) caused by proximity to the Gulf Stream. This may mitigate the biological "need" for extensive seasonal inshore-offshore movement by *H. gammarus*.[8]

There is an apparent affinity between the migratory group of lobsters east of Cape Cod, which are examined in this study, and those from Georges Bank and southern offshore canyons. Our evidence for movement of these lobsters north of Cape Cod into the Gulf of Maine seasonally, implies that genetic interchange between stock units continues, despite high exploitation rates. In light of this, management of fishing mortality rates on the offshore resource becomes an issue of increasing importance.

There is also increasing information on movements of lobster larvae, the distribution and behavior of newly settled and juvenile lobsters, concentrations of egg-bearing females, and the occurrence of long-distance homing behavior in American lobster both in the northern Gulf of Maine and southern New England waters. Some progress has been made in roughly delineating stock structure with the help of larval dispersion, hydrodynamic, and migration studies (NEFSC, 1993). Interpretation of these data, however, is tentative because migratory habits of larger lobsters appear extensive and may transcend the boundaries that some researchers attempt to draw solely on the basis of larval distribution. Despite many years of larval and postlarval lobster monitoring, a definitive stock-recruitment relation has yet to be determined, although ecological knowledge has been enhanced. The relative importance of the offshore lobster resource to recruitment in shoaler waters of the Gulf of Maine or other areas must be assessed. We need to know the degree of interchange between the two lobster groups in order to refine stock assessments.

[8] Bannister, R. C. 1997. The Centre for Environment, Fisheries and Aquaculture Science, Lowestoft Laboratory, Pakefield Road, Lowestoft, Suffolk, England NR33 0HT. Personal commun.

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Daily variability in abundance of larval fishes inside Beaufort Inlet

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Abstract.—We measured the daily abundance of larvae of eight species of ocean-spawned, estuarine-dependent fishes to determine the effect of sampling frequency on the mean and variance estimates during larval immigration past a permanent sampling station inside Beaufort Inlet, North Carolina, mid-November 1991 to mid-April 1992. Species of interest were *Brevoortia tyrannus, Lagodon rhomboides, Leiostomus xanthurus, Micropogonias undulatus, Mugil cephalus, Paralichthys albigutta, P. dentatus,* and *P. lethostigma.* Our data suggest that sampling at intervals >7 days can lead to excessive variance in abundance estimates. For all species, abundance varied as much as an order of magnitude from night to night. Proportional residuals from polynomial models of the seasonal recruitment pattern for a given species were used to assess the potential influence of nine environmental variables on daily densities. Twenty-seven of 72 correlations of proportional residuals with environmental variables were significant ($P<0.05$). Proportional residuals were positively correlated with time after dusk for six of eight species and were negatively correlated with turbidity for five of eight species. However, interpretation of correlations must be done cautiously because a species' recruitment pattern may coincide with normal seasonal change in one or more environmental variables. Variability in transport of larvae, from offshore to near the inlet and then through the inlet to the station, probably influences species abundance at the sampling station more than locally acting environmental variables. Daily collections of *B. tyrannus* larvae provided otoliths (n=1,341) showing that a large number of younger larvae, averaging 55 days posthatch, arrived at the station in mid-March on the date of maximum observed daily density (160 larvae per 100 m^3).

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From 1985 to the present, weekly sampling has been conducted near Beaufort Inlet to collect fish larvae entering the estuary during fall, winter, and spring (Warlen, 1994). Such inlets provide locations for sampling larvae in order to assess potential year-class strength of ocean-spawned but estuarine-dependent species. Abundance, size, and age data on early larvae in the sea, on advanced larvae in the inlets, and on juveniles in the estuaries can be used to understand when significant events such as mortality or rapid growth occur during a species' early life history. To obtain accurate abundance, size, and age estimates from the population of recruiting larvae, appropriate sampling protocol must be employed (Morse, 1989; Davis et al., 1990). Errors resulting from sampling bias can arise when larvae selectively avoid the sampling device or when there is nonrandom spatial (patchy) distribution of the larvae (Wiebe and Holland, 1968). Decreasing the time interval between sampling and decreasing the distance between stations in ichthyoplankton surveys increases the resolution of temporal or spatial patterns of species with patchy pelagic egg and larval distributions at both microscale (Houde and Lovdal, 1985) and mesoscale levels (Rowe and Epifanio, 1994).

Studies within NOAA's Southeast Atlantic Bight Recruitment Experiment (SABRE) are attempting to measure fluxes of larval fishes across the continental shelf and through inlets into estuaries amid myriad cyclical phenomena that bear directly on the larvae's abundance (Govoni and Pietrafesa, 1994; Stegmann and Yoder, 1996). The purpose of our SABRE study was to estimate the daily variation in abundance data collected on eight species of larval fish that seasonally ingress past the permanent sampling station at Pivers Island inside of Beaufort Inlet in order to determine an optimum sampling frequency for future sampling protocols. For one of these species, *Brevoortia tyrannus* (Atlantic menhaden), which has been the focus species in SABRE studies, additional analysis was conducted on age and growth with specimens collected daily. For all species, we used daily abundance data to calculate the decrease in precision of our relative abundance estimates as the interval between sampling events increased. Daily collections of larvae also allowed us to measure changes in size (length) of all eight

species. Finally, environmental variables were tested for their correlation with abundance.

Materials and methods

Sampling location and period

The sampling station for larval fish abundance, located 2 km inside of Beaufort Inlet, North Carolina (34°43'N, 76°40'W), was a platform attached to a bridge over a 6-m-deep tidal channel adjacent to the Beaufort Laboratory at Pivers Island and has been the site of weekly larval fish sampling since the 1985–86 larvae ingress season (Warlen, 1994). We sampled every night, 20 November 1991 through 15 April 1992, a period that more or less encompasses the annual periods of recruitment of ocean-spawned estuarine-dependent larvae that pass through North Carolina inlets from autumn to spring.

Fish and environment sampling

Oblique tows (bottom to surface) of a 1-m diameter, 800-μ mesh net were used to sample the water column for larvae. Three consecutive 4-min tows were made at 15-min intervals during the time of predicted maximum flood-tide current. Sampling was conducted only between dusk and dawn and about 50 minutes later each successive night because of the advancing tide stage. Oblique tows through the entire water column were chosen over surface, bottom, or other single-depth tows to eliminate depth bias. Species of concern, including *B. tyrannus*, are reported to be distributed by depth even in shallow, well-mixed North Carolina inlets (Lewis and Wilkens, 1971; Hettler and Barker, 1993).

The net was deployed by paying out the winch cable as the net, pulled downstream by the tidal current, sank to the bottom. It was then retrieved obliquely through the water column. A depth sounder with a deck readout (Standard Communications DS20) was attached to the net frame to indicate that the net had reached the bottom of the channel. Tow volume was measured with a General Oceanics model 2030R flow meter. Average tow time was 4.0 minutes. The target tow volume was 100 m^3, and target net retrieval speed was 1 m/sec.

Data on several environmental variables were collected concurrently with biological sampling. Salinity and temperature measurements were taken with a Hydrolab H20 water quality multiprobe. Water clarity was measured with a Sea Tech 25-cm transmissometer with a 660-nm filter. Tidal current speed was measured with a Marsh-McBirney model 201 flow meter. Wind speed and direction data were obtained from the NOAA C-MAN station at Cape Lookout, 15 km SE of the larval sampling platform. Tidal amplitude data were obtained from a NOAA tide gauge located on Pivers Island.

Processing of larvae

After preservation in 70% ethanol, fish larvae were identified, counted, and up to 20 individuals of each species were indiscriminately selected for measurement of standard length. Ages and birth dates of all menhaden larvae retained for length measurements (1,341 individual fish) were determined by otolith daily increment counts (estimated age in days = increment count + 5) following the methods of Warlen (1992).

Data analysis

Abundance was calculated from the number of larvae caught per tow and water volume sampled (density=number × 100 m^{-3}). Densities per unit volume were calculated rather than densities per unit area because all published relevant abundance data on these species is per unit volume. Mean densities by species for each date sampled were determined by averaging the densities from the three tows taken on that date. Although we sampled every night, no data were available for 10 dates during the sampling period (Fig. 1, A–D). This problem is explained by the following example. On 14 December, sampling started at 2359 h and ended around 0100 h, 15 December. The next night sampling began at 0033 h, 16 December. Thus, sampling never began on 15 December. Sampling on 15 December at the time of maximum flood tide current would have occurred before sampling on 14 December had ended. The same situation occurred on nine other dates.

Seasonal mean densities were determined by averaging the daily densities during the interval when each species was caught, including dates when no individuals of the species were caught. Variations in mean daily densities and associated variance estimates were derived by "sampling" individual density data sets for each species at intervals of 2, 3, 4, 5, 7, 14, and 30 days and by then comparing these with the actual data set (1-day intervals between sampling). From this exercise, a mean and standard deviation was generated for each sampling scheme. A 2-day cycle, for example, beginning on 20 November and continuing every other day at day 1, 3, 5 etc., produced one daily mean and standard deviation, whereas a 2-day cycle beginning a day later on 21 November and continuing every other day at day

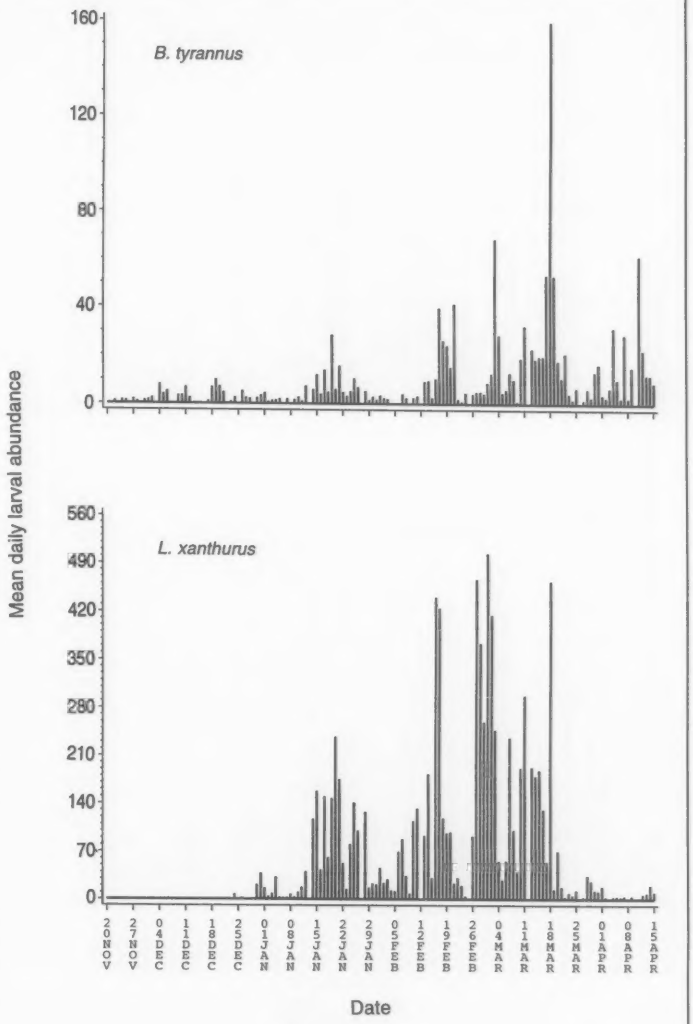

Figure 1A

Nightly mean densities (three tows each night) of larval Atlantic menhaden, *Brevoortia tyrannus*, and spot, *Leiostomus xanthurus*, caught near Beaufort Inlet, North Carolina.

2, 4, 6 etc. produced a different daily mean and standard deviation.

Spectral analysis (ARIMA procedure) was used to examine the time series of densities for each species for evidence of periodicities. Weekly density data based on two methods were compared by using a paired-mean Wilcoxon Signed Rank test. The Laird version (Laird et al., 1965) of the log-transformed Gompertz growth equation (Zweifel and Lasker, 1976) was used to describe the average growth of *B. tyrannus* larvae. The model was fitted to data for size and estimated age at time of capture. Log-transformed standard length (mm) and estimated age (in days) were used in the model (Warlen, 1992).

In order to examine the possible effects of environmental variables on observed larval densities, we first fitted polynomial regression models to the daily densities for each of the species' densities over time. Although a second-order polynomial was sufficient to describe the recruitment patterns of *Paralichthys*

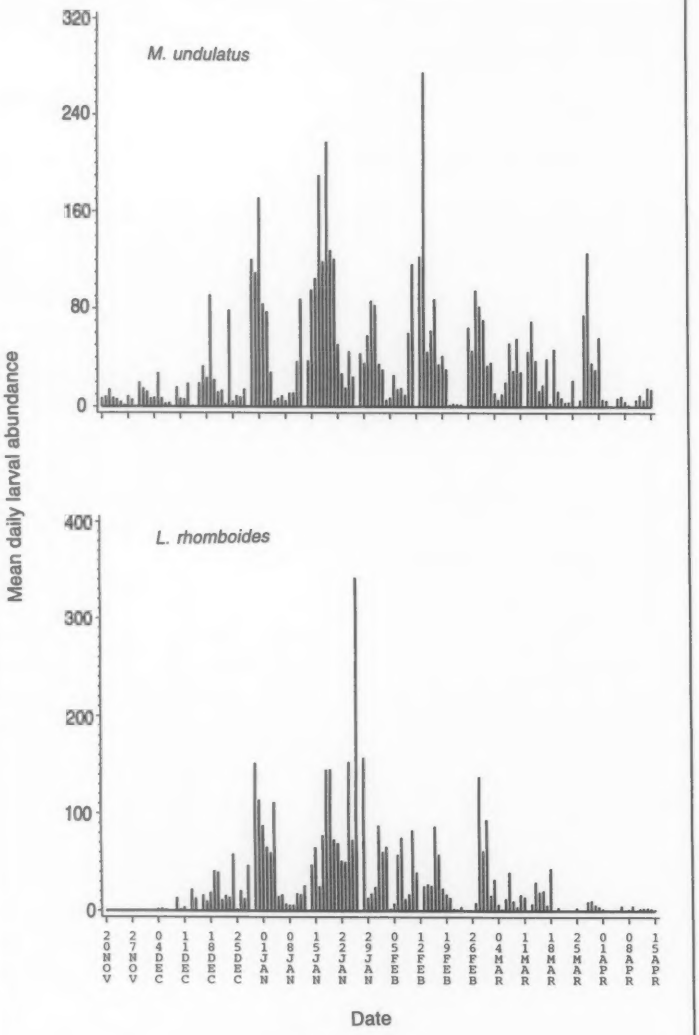

Figure 1B

Nightly mean densities (three tows each night) of larval Atlantic croaker, *Micropogonias undulatus*, and pinfish, *Lagodon rhomboides*, caught near Beaufort Inlet, North Carolina.

dentatus and *Micropogonias undulatus*, most species required a fourth-order polynomial, and *Brevoortia tyrannus* required a fifth-order polynomial. The polynomial models provided a means of estimating each species' density for each day as well as the difference between the observed density and the estimated density (residual). One would expect that if an environmental factor influenced observed density, it would do so in a proportional sense, i.e. its effects would be exhibited in relation to the expected density of the species at that date during the season of recruitment for that species. We therefore divided each residual by the expected density for that date to obtain a measure that took the species' recruitment pattern into account in looking at correlations with environmental variables. Durban-Watson statistics and first order autocorrelation coefficients were also computed to test the presence of autocorrelation and to measure its magnitude.

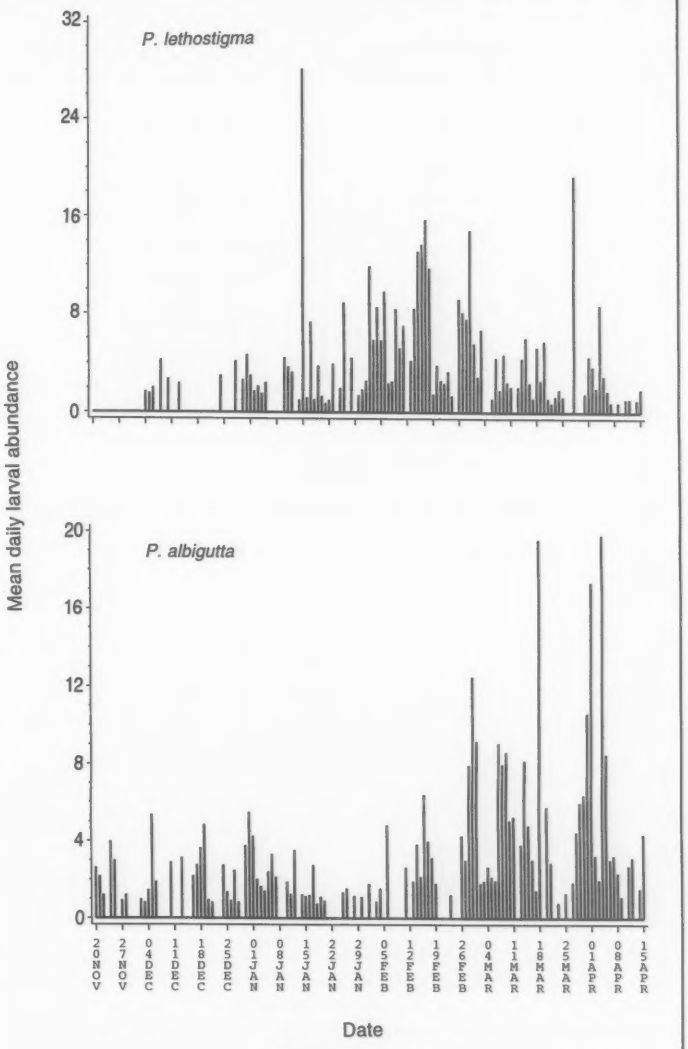

Figure 1C

Nightly mean densities (three tows each night) of larval southern flounder, *Paralichthys lethostigma*, and gulf flounder, *Paralichthys albigutta*, caught near Beaufort Inlet, North Carolina.

Results

Species abundance

The eight species selected for analysis accounted for 92% of the larvae caught during the period and are listed in Table 1 in order of decreasing abundance. Although abundance was not predictable from one night to the next for any species (Fig. 1, A–D), the Durban-Watson statistics for the polynomial regression models detected significant autocorrelation in the residuals for six of the eight species. However, one could not reject the null hypothesis that, for *Mugil cephalus* and for *Paralichthys albiguitta*, the test was inconclusive. The first-order autocorrelations for the other six species ranged from 0.30 for *Paralichthys dentatus* to 0.52 for *M. undulatus*. These reflect the serial dependency between densities on successive nights. On many dates, densities were two or more times less or more than the night

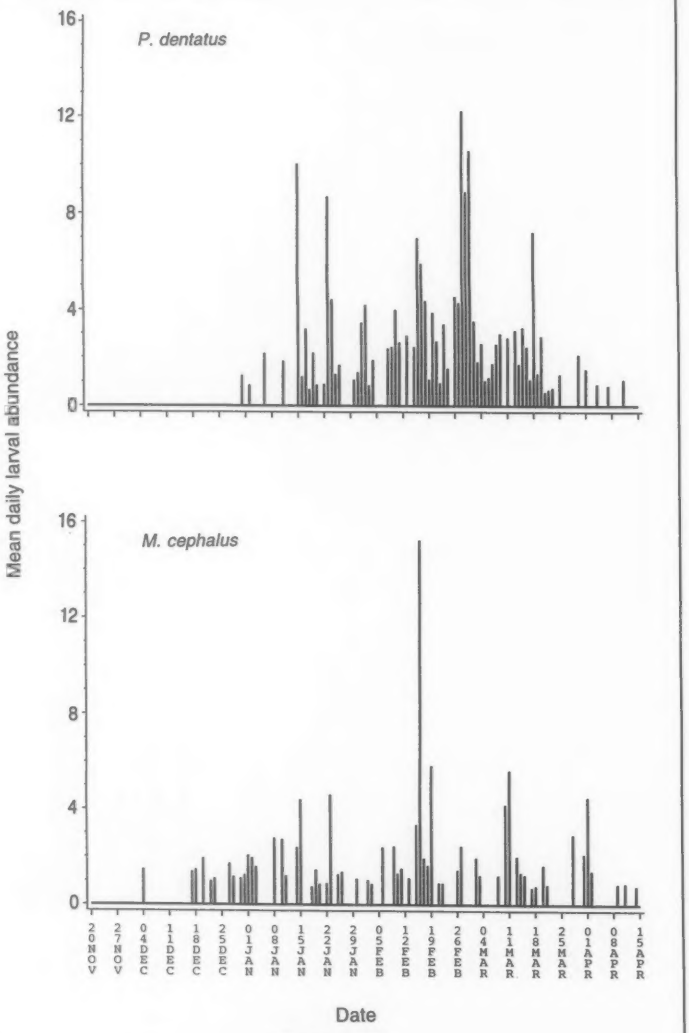

Figure 1D

Nightly mean densities (three tows each night) of larval summer flounder, *Paralichthys dentatus*, and striped mullet, *Mugil cephalus*, caught near Beaufort Inlet, North Carolina.

before; in some cases the difference between two consecutive nights was an order of magnitude (e.g, *Leiostomus xanthurus* on 18–19 March).

Although periods of low and high abundance can be seen along the time axis for each species, oscillations in abundance by each species did not occur at the same time in all cases. However, most of the species share periods of high abundance (mid January, mid February, early March, and mid March). We presumed that our data set would be a good candidate for time-series analysis. Spectral analyses of the estimated densities for each species was employed to reveal evidence of periodicities of varying length. All eight species exhibited a strong 14-day signal, most likely dominated by the lunar cycle. However, this 14-day signal turned out to be an artifact in the sampling method which was unavoidable. This artifact was due to sampling 50 min later each night in the sampling scheme (sampling at the same stage in the flood tide) until dawn, at which time the next sampling opportunity would be either 12 h 25 min later or 37 h 15 min later. We chose the later option (to

Table 1

Average seasonal density (=AveDen) and maximum daily density (=MaxDen) of target species listed in order of decreasing average density (larvae per 100 m^3). Sampling was conducted between 20 November 1991 and 15 April 1992.

Scientific name	Common name	AveDen	MaxDen	Capture date
Leiostomus xanthurus	spot	82.8	504.0	21 Dec–15 Apr
Micropogonias undulatus	Atlantic croaker	37.9	274.2	20 Nov–15 Apr
Lagodon rhomboides	pinfish	30.0	342.0	22 Nov–15 Apr
Brevoortia tyrannus	Atlantic menhaden	10.0	159.8	22 Nov–15 Apr
Paralichthys lethostigma	southern flounder	3.6	28.2	04 Dec–11 Apr
P. albigutta	gulf flounder	2.7	19.7	20 Nov–15 Apr
P. dentatus	summer flounder	2.0	12.2	31 Dec–15 Apr
Mugil cephalus	striped mullet	1.0	15.2	04 Dec–14 Apr

Table 2

Standard error of the mean abundance of larval species obtained at 2- to 30-day subsampling intervals of the actual daily sampling data set.

	If the number of days between sampling had been						
Scientific name	2	3	4	5	7	14	30
B. tyrannus	0.78	0.89	0.93	1.32	1.98	1.93	1.54
L. rhomboides	2.60	2.36	1.62	3.05	4.14	4.14	3.89
L. xanthurus	0.73	5.22	2.52	7.99	5.68	12.86	9.50
M. undulatus	1.96	2.28	1.17	1.37	1.90	5.90	3.65
M. cephalus	0.31	0.26	0.22	0.21	0.38	0.24	0.33
P. albigutta	0.37	0.14	0.44	0.19	0.27	0.26	0.31
P. dentatus	0.07	0.26	0.14	0.09	0.19	0.32	0.38
P. lethostigma	0.15	0.24	0.43	0.22	0.42	0.48	0.46

avoid sampling twice on the same date), but either option would have resulted in an irregular pulse interval in the time line every 14 days and would have precluded further time-series analysis.

Within-night variability

Variability between tows of the three tows each night was estimated by calculating the coefficient of variation (CV) for each night and for each species. The CV averaged about 50% for each species throughout the sampling period. Late in the immigration period, the CV in densities within a night increased for *Paralichthys lethostigma*; for all other species daily tow-to-tow variability was relatively constant throughout the time series.

Sampling interval

The range in density estimates derived from subsampling the actual data set increased as the subsampling interval increased (Fig. 2), as did the standard error of the mean for most species (except *Mugil cephalus* and *Paralichthys albigutta*) (Table 2). For example, sampling every night yielded a seasonal mean of 10 *B. tyrannus* larvae per 100 m^3, but if we had sampled only every 30 days our estimate for the 1991–92 immigration season, seasonal mean abundance could have ranged from 3 to 43 larvae per 100 m^3, depending on which date we began sampling. Similarly, had we sampled for *L. xanthurus* every 30 days, our estimated seasonal mean could have ranged from 11 to 249 larvae per 100 m^3.

Daily versus weekly sampling

Sampling methods were compared to see if the increased effort required for daily sampling provided better abundance estimates than did weekly sampling. Weekly estimates were calculated by using *B. tyrannus* abundance data from our daily 1-m-net oblique-tows and are plotted (Fig. 3) along with

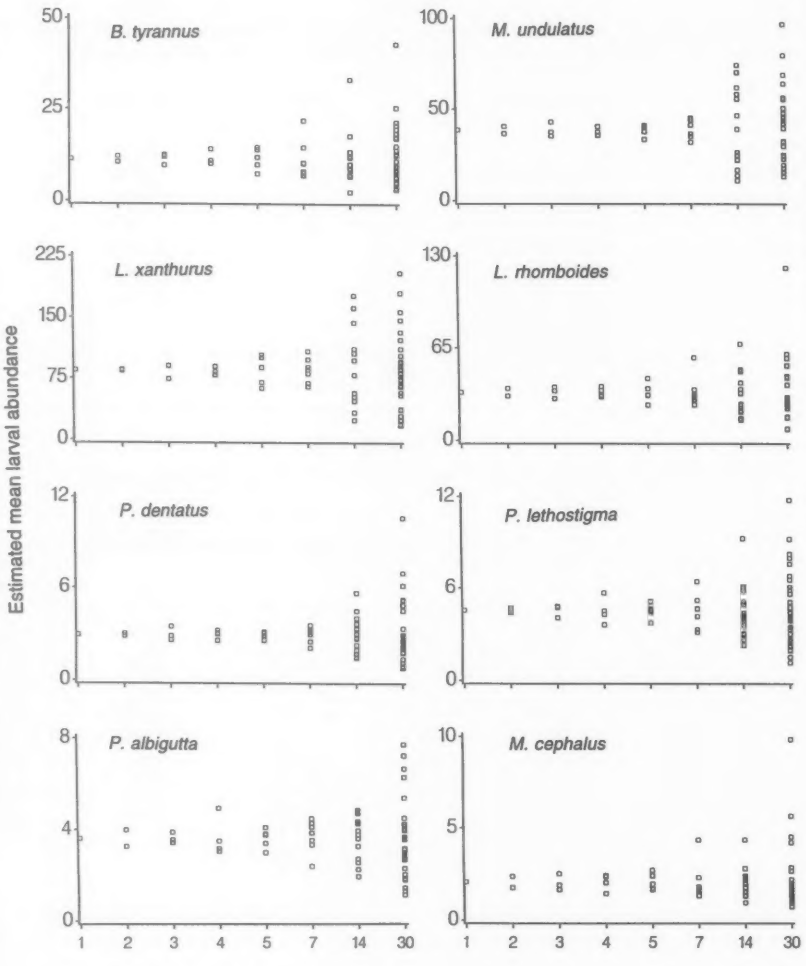

Figure 2

Estimated effect, based on daily sampling data, of sampling interval on estimated larval fish abundance.

B. tyrannus data obtained with a 1 × 2 m net fished near the surface (Warlen, 1994). Sampling with a 1 × 2 m surface net occurred at night, at approximately the same hour, from the same sampling platform, and during the same immigration season (1991–92) as our basic study. The seasonal weekly mean of the weekly means calculated from daily means obtained with the 1-m net was significantly different ($P<0.05$) from the seasonal weekly mean density obtained with the 1 × 2 m surface net. Sampling daily with the 1-m oblique net resulted in an average of 10.0 *B. tyrannus* larvae per 100 m^3 compared with 7.2 larvae in weekly sampling during the same season with the 1 × 2 m surface net (Warlen, 1994). The abundance of *B. tyrannus* in catches of the 1-m oblique net tows made only on the nights that the 1 × 2 m surface net was fished appeared similar to the weekly average of the daily catches with the 1-m oblique net tows (10.2 vs. 10.0 larvae per 100 m^3), but this similarity could not be statistically tested because the samples were not independent.

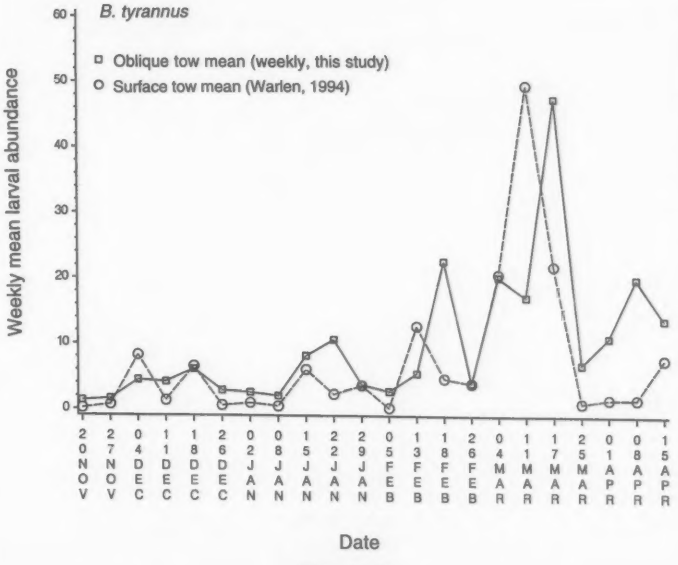

Figure 3

Comparison of mean densities from nightly sampling (oblique tows, 1-m-diameter net) averaged by week with mean densities estimated from Warlen's (1994) weekly sampling (surface tows, 1 × 2 m net).

Size of larvae

Plots of mean length for the larval species produced a variety of seasonal patterns (Fig. 4). Although *B. tyrannus* increased in average size until mid-March and then decreased, the mean length of *M. undulatus, L. xanthurus,* and *Lagodon rhomboides* peaked in mid-February, then decreased. *M. undulatus* seemed to share peaks in abundance with peaks in increasing mean lengths (e.g. 30 December, 17 and 26 January, and 28 February). Larvae of *Paralichthys* or *Mugil* did not change substantially in length over the season.

Age and growth of menhaden larvae

Because SABRE studies have centered around *B. tyrannus*, daily collections of this species provided a unique opportunity to test correlations of observed larval age structure with waves of immigrating larvae and environmental conditions. Otoliths of *B. tyrannus* (10–32 mm SL) showed a range in estimated age of 16 to 106 days (Fig. 5). The Gompertz growth equation predicted a size at hatching of 4.96 mm SL, which is above the reported size at hatching of 3.2–3.4 mm SL for laboratory-reared specimens (Powell, 1993). Average daily growth rate declined from 0.32 mm/day between days 30 and 40 to 0.03 mm/day between days 80 and 90.

According to back calculations from capture date, *B. tyrannus* ingressing Beaufort Inlet spawned from 12 October 1991 to 16 February 1992. Expressed as a percentage of the total Atlantic menhaden caught, two age cohorts, one in mid-December and another in late January, made up about 50% of the year's recruitment (Fig. 6). Almost 5% of the Atlantic menhaden larvae captured at the sampling station during the season were hatched on 13 December 1991.

The distribution of estimated ages of larvae by collection date is shown with an overlay plot of mean daily density (Fig. 7). The largest daily mean density of 159 larvae per 100 m^3 (18 March 1992) occurred during a decrease in age distribution of about 25 days and would suggest a significant import of a younger cohort of *B. tyrannus*.

Environmental variables

The environmental conditions (except barometric pressure) recorded at the time of sampling are shown in Figure 8. Spearman correlation coefficients were computed for each of the eight species with each of the nine environmental variables (Table 3). Twenty-seven of the 72 coefficients were significant ($P<0.05$).

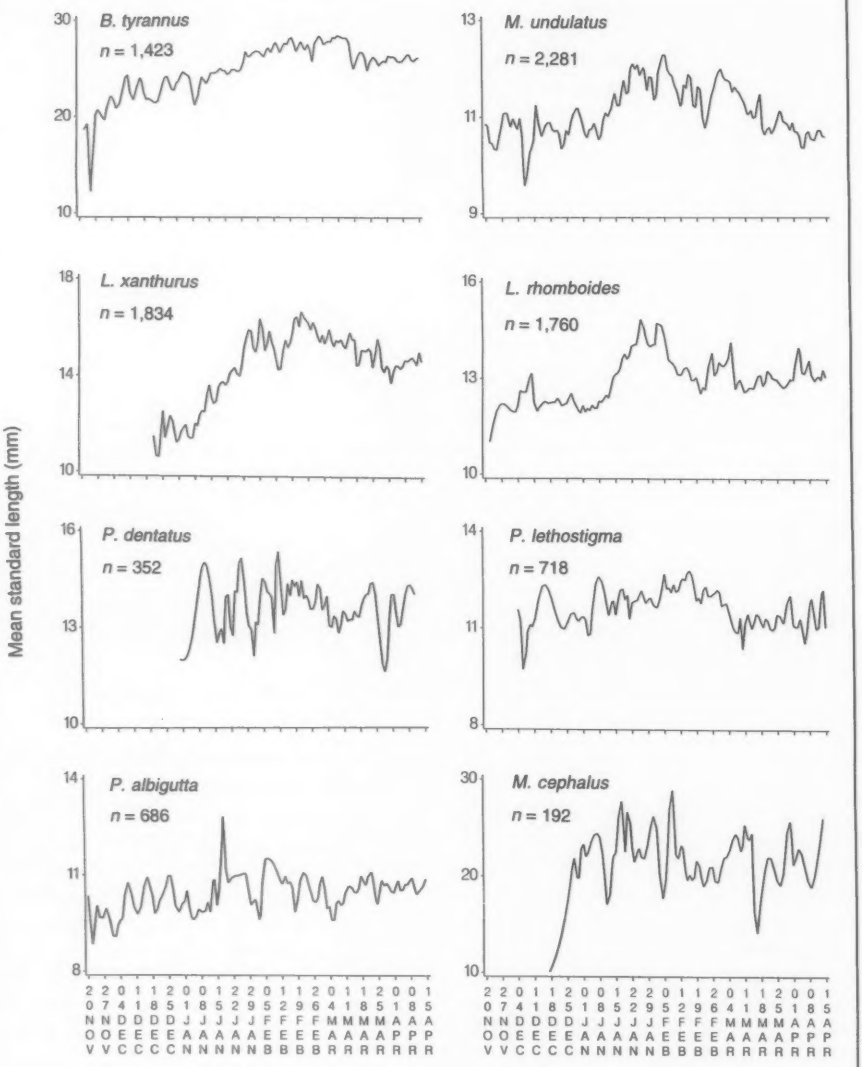

Figure 4

Standard lengths of each species; n = number of specimens measured.

The proportional residuals were positively correlated with hour of capture after dusk for six species and negatively correlated with turbidity for five species. As an example, the regressions of abundance of *B. tyrannus* on each environmental condition are shown in Figure 9. Significant correlations for this species were found with tidal amplitude, current speed, moon phase (=spring vs. neap tide), water clarity, and hours after dark when sampling occurred. *Paralichthys dentatus,* however, exhibited no correlations.

As would be expected, there were significant correlations between the nine environmental variables. The highest was between tidal amplitude and surface current (0.60), and the next highest was between atmospheric pressure and wind velocity (–0.44). However a principal components analysis yielded four

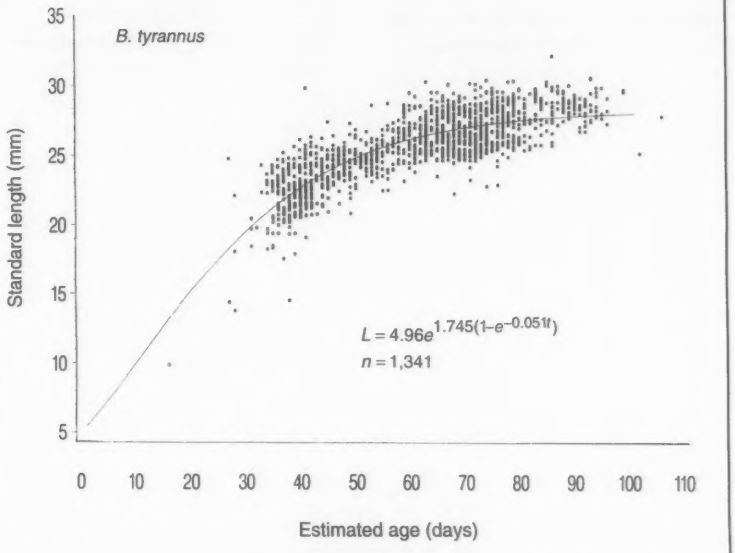

Figure 5

Growth of *B. tyrannus* larvae as described by the Gompertz model where L = standard length in mm and t = estimated age in days. Dots represent length-at-age measurement on each larva.

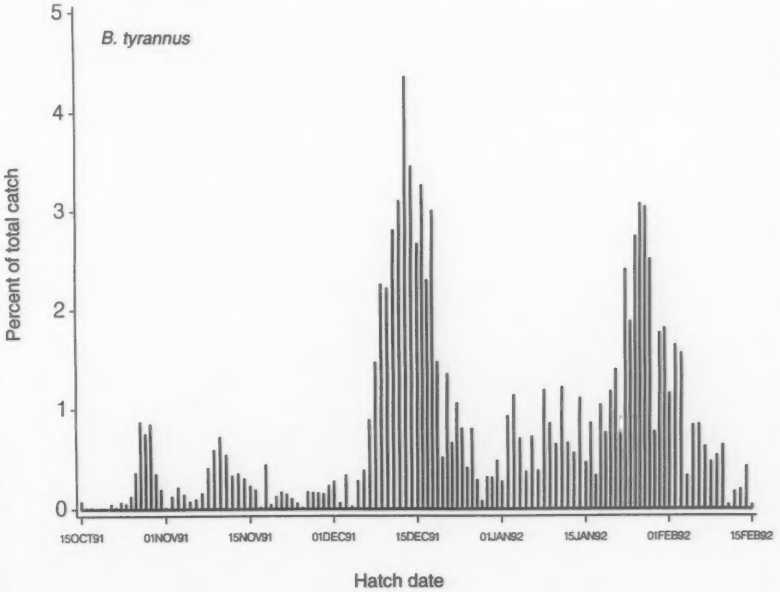

Figure 6

Density-weighted percentage of *B. tyrannus* larvae ingressing through Beaufort Inlet by back-calculated hatch date.

eigenvalues greater than 1.0, cumulatively accounting for only 66% of the variation, leading us to conclude that the structure of the environmental data was not simple.

Discussion

The periodic nature of seasonal changes in some environmental variables and in reproduction of the dif-

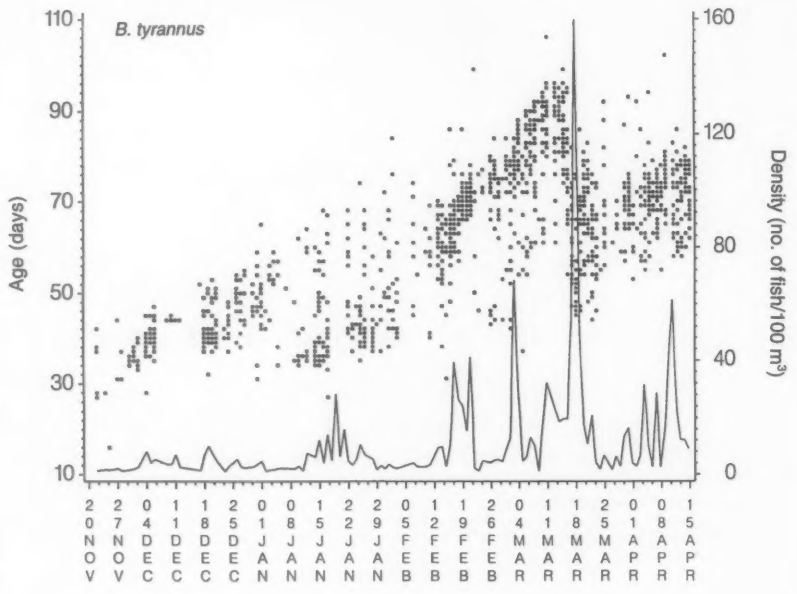

Figure 7

Distribution of estimated ages (points, left axis) and mean daily abundance (line, right axis) of *B. tyrannus* larvae collected throughout the sampling period.

Table 3

Spearman correlation coefficients between proportional residuals for species' densities and various environmental variables: amplitude = tidal amplitude; pressure = barometric pressure; current = flood tide current; hours = hours after sunset; spring tide = spring tide (versus neap tide); temperature = water temperature; wind velocity = average daily wind speed. Bt=*B. tyrannus*, Lr=*L. rhomboides*, Lx=*L. xanthurus*, Mu=*M. undulatus*, Mc=*M. cephalus*, Pa=*P. albigutta*, Pd=*P. dentatus*, Pl=*P. lethostigma*. *=$P<0.05$. **=$P<0.01$. ***=$P<0.001$.

Variable	Bt	Lr	Lx	Mu	Mc	Pa	Pd	Pl
Amplitude	0.40***	0.19*	0.12	0.08	–0.24	–0.02	0.20	0.08
Pressure	0.11	–0.09	0.04	0.03	–0.24	–0.08	–0.02	–0.20*
Current	0.29***	0.22*	0.06	0.14	–0.11	0.15	0.10	0.20*
Hours	0.19*	0.30***	0.30**	0.43***	0.01	0.24**	0.17	0.22*
Salinity	0.06	0.01	–0.20*	–0.11	–0.08	–0.18*	0.14	–0.19*
Spring tide	–0.26**	0.04	–0.22*	–0.24**	–0.00	0.00	–0.19	0.09
Temperature	–0.07	–0.24**	–0.05	–0.06	0.18	0.02	0.08	0.00
Turbidity	–0.28***	–0.42***	–0.32***	–0.29***	0.10	–0.19*	0.03	–0.17
Wind velocity	0.14	0.28*	0.22*	0.06	0.31*	0.11	0.16	0.15
(Sample size)	133	116	102	133	61	138	91	125

ferent species implies that care must be taken in the interpretation of correlations between these variables and fish densities. For example, temperature may be inappropriate to associate with observed densities owing to the spawning-season periodicities involved with life history strategies of each species. Time of spawning (e.g. early winter) and the arrival at the inlet after a 2–3 month cross-shelf transport time, could result in higher abundance corresponding with rising temperatures. The multiple-regres-

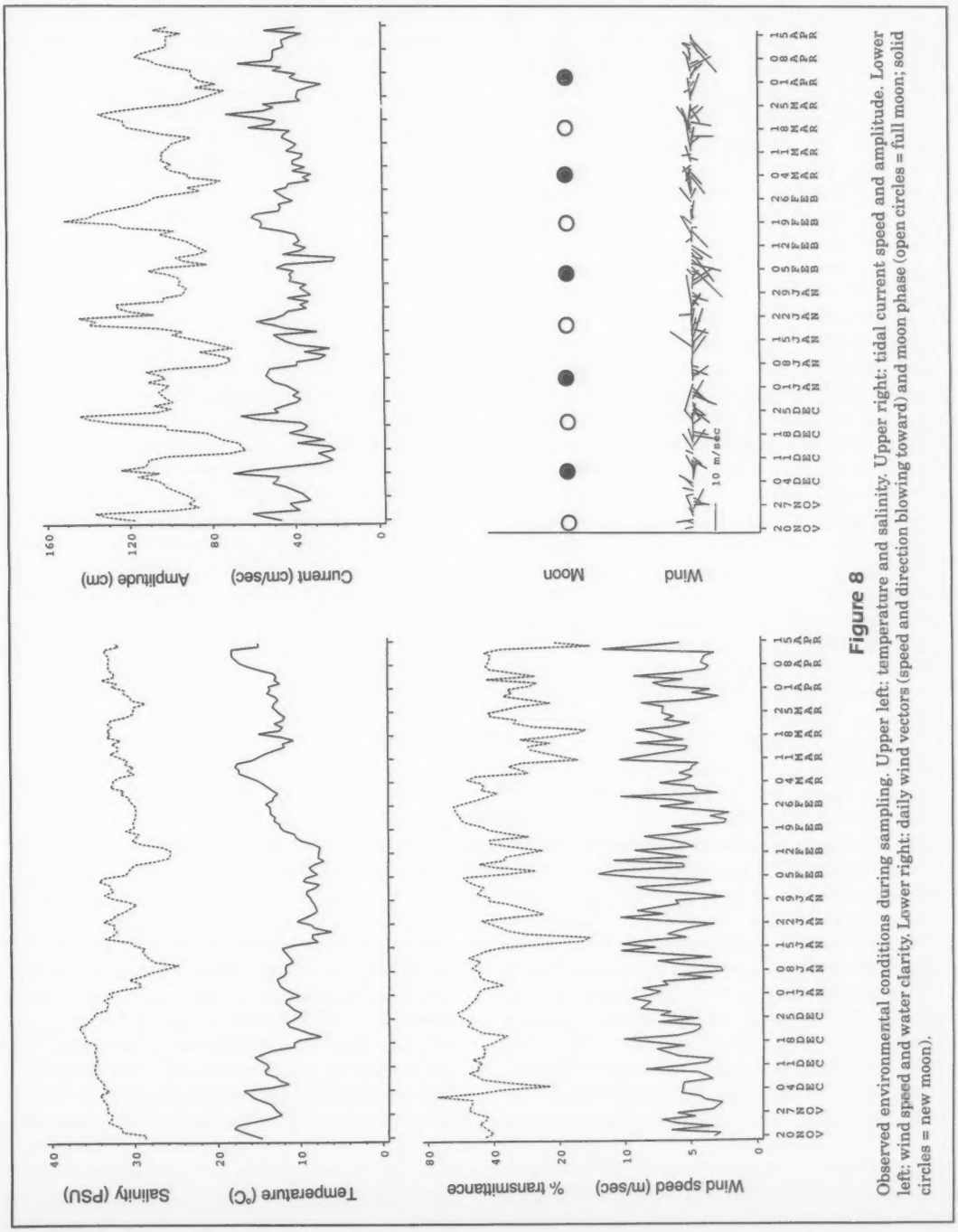

Figure 8

Observed environmental conditions during sampling. Upper left: temperature and salinity. Upper right: tidal current speed and amplitude. Lower left: wind speed and water clarity. Lower right: daily wind vectors (speed and direction blowing toward) and moon phase (open circles = full moon; solid circles = new moon).

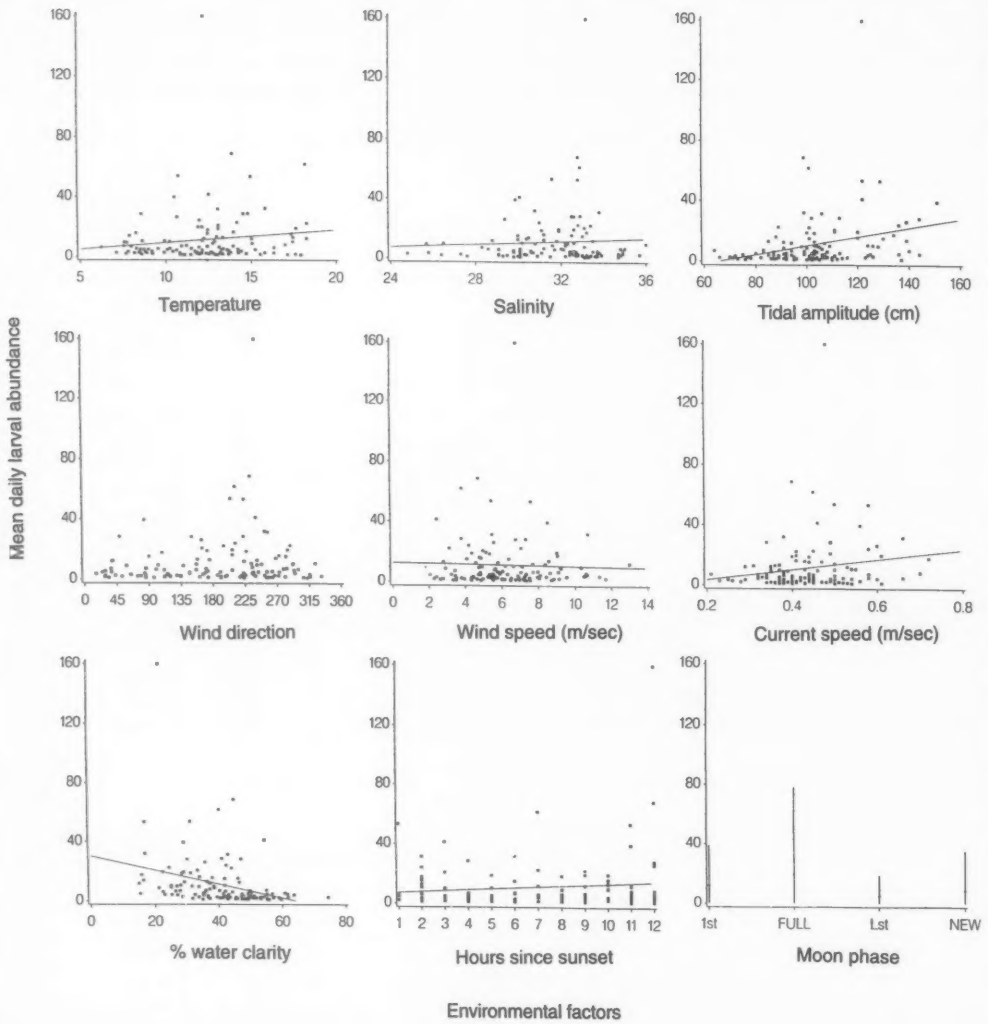

Figure 9

Abundance of *B. tyrannus* collected during 138 sampling nights versus nine environmental factors. Wind direction is direction wind is coming from. Vertical axis is abundance (number larvae per 100 m^3).

sion analysis and the principal-component analysis suggested that unknown factors on a scale larger than the locally measured environmental conditions are probably more important in causing peaks in abundance of the immigrating larvae. Originating from wide-spread spawning sites during the fall-winter period, larvae of different spawning cohorts arrive near the inlet from many possible transport routes. The most likely factor is onshore displacement of warm Gulf Stream filaments containing patches of larvae (Stegmann and Yoder, 1996). Once under the influence of tidal exchange at the inlet, patches of larvae merge as they ingress the estuary. Variables including temperature, salinity, turbulence, turbidity, odors, and currents differentially affect survival or active transport behavior into the estuary (Boehlert and Mundy, 1988). Larval behavior may have contributed to the fact that some species were more abundant in catches made later in the night (e.g. *L. rhomboides, L. xanthurus, M. cephalus,* and *P. dentatus*), acting to disperse larvae into the water column from the edges and bottom, thus making them more vulnerable to the sampling gear.

Daily ageing of *B. tyrannus* caught during the study revealed that a rapid and distinct shift in larval populations occurred in mid-March (Fig. 7). In early March, the recruiting larvae were primarily from a cohort that was spawned in mid-December, first reached the inlet in mid-January, and suddenly disappeared on 16 March. On 18 March a new cohort, spawned in mid-January, appeared. Its appearance was coincident with the year's highest daily mean density. This change in age structure coincided with a 3-day shift to southwesterly winds and full-moon spring tides. Advanced very high resolution radiometer sea surface temperature (AVHRR-SST) imagery revealed that sea surface temperatures 15 km off Beaufort Inlet warmed to 15°C on 9 March 1992, up from 11°C a week earlier (and down to 12.3°C a week later), possibly bringing in the younger *B. tyrannus* larvae from warmer offshore water to the vicinity of Beaufort Inlet (Stegmann[1]). This warming was also detected by our temperature measurements at the sampling platform, rising to 17.9°C on 9 March followed by a decrease to 12°C on 18 March (Fig. 8), when the large number of younger larvae were caught. Until that date, the average estimated age of larvae caught in Beaufort Inlet increased from about 35 days in late November to about 80 days in mid-March. About 57% of the menhaden captured in the 1991–92 season were spawned in two 2-week periods (mid-December and late January). Although substantial spawning may have occurred at other times, the population from which these larvae were caught contained the survivors of the cross-shelf transport. It appears that size at estimated age was significantly larger, about 3 mm SL, for ages between 40 and 80 days for the 1991–92 collections than the larval size reported by Warlen (1992) for *B. tyrannus* collected mainly offshore of Beaufort Inlet in 1979–80. Because the daily ageing method was the same, this observed difference in growth rates of the 1991–92 ingressing larvae is attributed to a higher growth rate among the larvae that survive to reach the inlet.

Coastal marine environments experience periods of diurnal or semidurnal tidal cycles imbedded within lunar and semilunar cycles, and these have been shown to influence a broad range of organisms and processes (Hutchinson and Sklar, 1993). Cyclical phenomena impose a particular set of requirements for their adequate measurement and for the avoidance of bias arising from aliasing (Kelly, 1976) because the temporal sequence of observations will be autocorrelated. A circannual rhythm is a feature of the life history of most vertebrates, and one manifestation of this is the restriction of reproduction of a species to a season of several weeks or months. If the purpose of a sampling program is to compare recruitment of a species of larval fish from year to year, then it should be designed so that it describes each year's temporal pattern accurately. In this respect it differs from the normal random sampling situation designed to estimate the mean and variance of some statistical population. Because its purpose is to enable description of a temporal pattern, a systematic design is normally chosen to ensure equal (or near equal) spacing of samples. Equal spacing of samples is required for many approaches for the analysis of a time series. However, if there are other, shorter cycles within the seasonal pattern, then care must be taken to avoid spurious results (aliasing) that can arise when the sampling interval is greater than one half the wavelength of a significant component cycle. Sampling to determine a seasonal flux of larvae should be designed to detect temporal patterns, as well as estimate a mean abundance and variance.

The question of sampling frequency may have a lower priority than considerations of sampling costs and vessel availability, and thus it is important to quantify the effect that sampling frequency has on estimates of larval abundance, size, and age. For example, one may be interested in estimating the flux of larvae across a boundary over some unit of time. If one is interested in the strength of a year class, the sampling effort must include the entire season of larval recruitment of that species. If one is interested in evaluating the influence of a meteorological event on larval distribution, then the appropriate sampling interval would be measured in hours. As we shall see, both sampling designs also require due consideration of various physical and biological rhythms that have an important bearing on the number of larvae collected at a given point in space and time (e.g. tidal, circadian, circannual). Prior to establishing sampling protocol for future studies, we attempted to determine a sampling interval that would provide acceptable larval fish abundance estimates. Larval fish surveys have been made in Beaufort Inlet and other North Carolina inlets on different sampling intervals, i.e. weekly (Warlen, 1994), bi-weekly (Lewis and Mann, 1971), every new and full moon period (Hettler and Chester, 1990) and every new moon period (Hettler and Barker, 1993). When we compared the weekly sampling method that has been used for monitoring at Pivers Island for the past 10 years (Warlen, 1994) with our daily sampling experiment, a difference in estimated abundance of *B. tyrannus* was detected. Differences in the two types of nets may have

[1] Stegmann, P. 1996. Graduate School of Oceanography, Univ. Rhode Island, S. Ferry Road, Narragansett, RI 02882. Personal commun.

been responsible for the differences in catches. The 1-m net is an active gear, retrieved obliquely at a rate of about 1 m/sec through the water column, whereas the 1 × 2 m net is fished passively in the surface current (flowing 0.2–0.5 m/sec, Warlen[2]). Also, the mesh of the 1-m net is 200 m smaller and may have reduced extrusion of larvae. Density estimates derived from sampling one night per week with the 1-m net were similar to estimates derived from sampling every night per week with the 1-m net made on the same night as the 1 × 2 m surface net sets and further support our suspicions regarding the differences in sampling with the two gears. Finally, we have assumed for 10 years (Warlen[2]) that the Pivers Island station serves as a proxy for Beaufort Inlet. An intensive SABRE study conducted in March 1996, during which larval fish were sampled synchronously at seven locations in and near the inlet, including Pivers Island, is under analysis to determine how closely Pivers Island larval fish densities reflect Beaufort Inlet densities.

We conclude that at least one sampling event each week is required for estimating late autumn through early spring seasonal abundance of fish larvae in North Carolina inlets, although more frequent sampling is preferred if the standard error of estimates is to be reduced. Bias may be introduced by sampling only at a specific period of the lunar cycle, because larval abundance appeared to decrease following spring tides. Studies where sampling is done exclusively at the same lunar phase may consistently over or under estimate abundance. This attribute may be acceptable for interannual comparisons, if the same methods are followed from year to year, but would not be acceptable for calculating the flux of larvae through an inlet. Sampling at intervals of 7 days or less can reduce this bias.

Acknowledgments

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Effects of geography and bathymetry on growth and maturity of yellowfin sole, *Pleuronectes asper*, in the eastern Bering Sea

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Abstract–Length at age, length at maturity, and age at maturity of yellowfin sole, *Pleuronectes asper*, in the eastern Bering Sea, are influenced by area of sampling and bottom depth. Yellowfin sole sampled during spring and summer bottom trawl surveys (1982–94) grew faster in the northwest area compared with the southeast area. Mean lengths at age were generally more than 2 cm greater than those for southeast fish at ages greater than 10 years. Length at 50% maturity in females during 1993 and 1994 was respectively 2.3 cm and 0.94 cm larger in the northwest area than in the southeast area. In contrast, there was no apparent difference in age at 50% maturity between areas.

Spring-summer patterns in bathymetric habitation of yellowfin sole differ for immature and mature individuals and cause a potential bias in estimates of growth and maturity. There is a clear relation between length and depth for immature fish, with older, immature fish inhabiting deeper water. In contrast, mature fish distribute similarly by size across a wide range of bottom depths. As a result, estimates of length and age at 50% maturity (L_{50}, A_{50}) tended to increase with increasing bottom depth. Because current resource assessment surveys do not sample the shallowest areas of the summer distribution of yellowfin sole, estimates of L_{50} and A_{50} are inherently biased high.

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Fishery Bulletin 95:494–503 (1997).

Yellowfin sole, *Pleuronectes asper*, inhabit the nearshore shelf areas of the eastern Bering Sea, from Bristol Bay to north of Nunivak Island (60°N lat.)(Fig. 1), during spring and summer months. Adult individuals overwinter near the shelf-slope break at approximately 200 m. Two main eastern Bering Sea overwintering groups, composed mainly of sexually mature adults (Krivobok and Tarkovskaya, 1964) have been identified: a southern complex near Unimak Island and a central complex located west of the Pribilof Islands (Fadeev, 1970; Bakkala, 1981; Wakabayashi, 1989). During spring, as the edge of the shelf ice recedes toward the coast, yellowfin sole migrate across the shelf to nearshore spawning areas in Bristol Bay and off Nunivak Island (Bakkala, 1981). Yellowfin sole generally spawn at bottom depths less than 50 m (Wilderbuer et al., 1992); most spawning activity, however, occurs at depths less than 30 m, May through August (Nichol, 1995). Juvenile yellowfin sole probably do not undergo the long cross-shelf migration. At least some juveniles (<6 years) are known to overwinter nearshore (Fadeev, 1970; Wilderbuer et al., 1992), whereas relatively few juveniles overwinter offshore (Krivobok and Tarkovskaya, 1964; Fadeev, 1970).

Bottom trawl surveys for groundfish resource assessment are conducted annually in the eastern Bering Sea to obtain abundance estimates of commercially important fish and invertebrate species. Differences in fish distributions and oceanographic factors have prompted scientists who analyze survey results to stratify the eastern Bering Sea into discrete northwest and southeast areas, and three different depth regimes (Walters and McPhail, 1982; Walters, 1983; Wakabayashi, 1989; Bakkala, 1993). Thus, both geographic and bathymetric factors affect fish distribution and abundance in the eastern Bering Sea. In this paper I describe regional differences in growth of yellowfin sole (*P. asper*) from the eastern Bering Sea and effects of geographic area and bottom depth on estimates of length and age at maturity.

Commercial catch records (Norris et al.[1]) indicate that yellowfin sole occur in substantial numbers in waters shallower than 30 m, where

[1] Norris, J. G., J. D. Berger, and K. T. Black. 1991. Fisherman's guide to catch per unit effort and bycatch data from the National Marine Fisheries Service Observer Program: Bering Sea/Aleutian Island yellowfin sole trawl fishery. AFSC Proc. Rep. NOAA-NMFS 91-07, 200 p. Alaska Fisheries Science Center, Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle, WA 98115-0070.

research surveys are not routinely carried out. Thus, current survey biomass estimates underestimate population abundance of the species; exclusion of juveniles in shallow water, as well as sexually mature yellowfin sole that inhabit these nearshore spawning areas during the survey period (June–August), may introduce a sampling bias into estimates of growth and estimates of size at maturation. To determine the extent of this potential problem, I considered the effects of bottom depth on fish size.

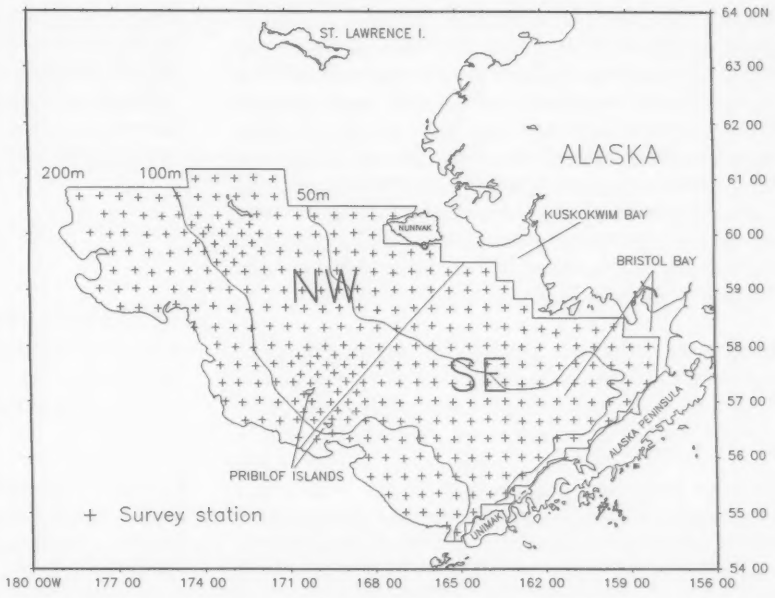

Figure 1

Map of the survey stations where yellowfin sole were sampled by the eastern Bering Sea crab-groundfish bottom trawl survey of the Alaska Fisheries Science Center from 1982 to 1994. Northwest (NW) and southeast (SE) areas are delineated by the strata boundary line extending from north of Kuskokwim Bay, southeast through the Pribilof Islands.

Materials and methods

Survey area

Resource assessment surveys were conducted in the eastern Bering Sea (Bakkala, 1993; Wakabayashi et al., 1985), from June to mid-August. Standard survey stations were based on a 20 by 20 nautical mile grid that covered the area from inner Bristol Bay west to the continental slope edge and from the Alaska Peninsula north to approximately latitude 61°N (Fig. 1). Bottom trawl tows of approximately 1.5 nautical miles and of 30-min duration were made at each station. Surveys began in inner Bristol Bay and generally followed north- and south-directed transects, proceeding westward with each finished transect.

Data

Yellowfin sole otoliths were collected from 3,891 males and 5,209 females during AFSC surveys, 1982–94 (Table 1). Fish were measured to the nearest centimeter total length (TL) and sagittal otoliths were removed and stored in 50% ethanol for subsequent age determination. Ages were determined by using the break-and-burn technique (Chilton and Beamish, 1982).

Female maturity data were collected during 1992–94 surveys (Table 2). Maturity codes were based on macroscopic gonadal appearance (Nichol, 1995). For the purpose of this study, codes were simplified to either mature or immature. Females were considered mature if ovaries contained yolked or hydrated oocytes, or were recently spent. Immature ovaries contained no visible oocytes. Data collected during 1993 (Table 2) were best for examining spatial differences in female length at maturity because samples were collected at nearly all survey stations within the 50-m contour line, and maturity code assignments were verified by histological examination (Nichol, 1995).

Analysis

The total survey area was subdivided into northwest and southeast areas (Wakabayashi, 1989; Bakkala, 1993) extending from north of Kuskokwim Bay southeast through the Pribilof Islands (Fig. 1).

Length at age Factorial analysis of variance (ANOVA) models (Reish et al., 1985) was constructed independently for males and females to examine the variance in length at each age due to geographic area (northwest or southeast) and to bottom depth. For ANOVA, bottom depths were grouped into three levels (<30 m, 30–49 m, and ≥50 m; Table 1). A model, which included interaction terms and which was pooled across years, was analyzed for both males and

females. Preferred models were estimated by a least-squares backward stepwise procedure that sequentially removed the highest-order nonsignifcant factor until only significant terms remained. Nonsignificant ($P \geq 0.05$) main effects were retained if they were included in one or more significant interaction terms. Residual plots were examined to test assumptions of homoscedasticity and normality of error terms. Least squares estimates of model coefficients were obtained by means of the Statistical Analysis System procedure GLM (SAS Institute, 1989).

Length at maturity Logistic regression was used to assess the effects of area and bottom depth on the probability that an individual of a given length (cm) was mature or immature. The following equation was fitted independently to 1992, 1993, and 1994 female yellowfin sole length-maturity data by using the Statistical Analysis System maximum likelihood procedure LOGISTIC (SAS Institute, 1989):

$$MAT = \frac{1}{1+e^{-(\mu+\beta \cdot L+\alpha \cdot area+\delta \cdot D+\Delta \cdot L \cdot D)}}, \quad (1)$$

where MAT = mature proportion of female yellowfin sole given its length (L), area, and bottom depth (D) of capture.

Area was treated as a factor indicating either northwest or southeast areas. Depth was treated as a continuous variable. Length, area, and depth coefficients were represented by β, α, and δ, respectively, and μ denoted the intercept (on the logit scale). A length × depth interaction term ($L \cdot D$; Eq. 1) with coefficient Δ was also tested for significance. Nonsignificant ($P \geq 0.05$) highest order terms were removed from the model.

Length at 50% maturity (L_{50}) was calculated by substituting 0.5 for MAT in Equation 1 and solving for L as follows:

$$L_{50} = -\left(\frac{\mu+\alpha \cdot area+\delta \cdot D+\Delta \cdot L \cdot D}{\beta}\right). \quad (2)$$

Due to sample-size inequalities (Table 2), length at maturity comparisons between northwest and southeast areas were limited to females sampled in 1993 and 1994.

Table 1

Number of male and female yellowfin sole sampled for age and length by the Alaska Fisheries Science Center during resource assessment surveys conducted in northwest and southeast areas of the eastern Bering Sea from 1982 to 1994.

	Bottom depth (m)					
	Northwest			Southeast		
Year	<30	30–49	≥50	<30	30–49	≥50
Males						
1982	26	58	26	65	97	45
1983	0	52	85	56	87	27
1984	14	102	31	31	109	42
1985	28	99	36	26	65	84
1986	0	100	42	39	74	66
1987	133	30	13	38	111	31
1988	44	33	13	27	28	98
1989	29	35	39	23	105	87
1990	49	58	48	36	74	92
1991	82	64	26	0	65	97
1992	32	0	25	73	79	39
1993	31	50	18	47	0	46
1994	24	64	21	72	13	37
Total	492	745	423	533	907	791
Females						
1982	15	110	49	65	97	83
1983	0	80	129	57	102	41
1984	0	143	71	33	137	83
1985	41	154	40	43	57	129
1986	0	113	97	48	57	103
1987	152	67	24	36	112	51
1988	58	38	46	47	31	100
1989	0	82	113	39	92	96
1990	47	81	86	54	38	129
1991	85	73	35	0	69	146
1992	35	0	91	86	79	67
1993	28	67	100	61	0	101
1994	29	55	49	61	20	76
Total	490	1,063	930	630	891	1,205

Table 2

Summary of female yellowfin sole length-maturity collections during the 1992–94 Alaska Fisheries Science Center eastern Bering Sea groundfish bottom trawl surveys. A = Age, length, and maturity data collected by sex-cm interval; L = Length and maturity (random measurements); O = Ovaries, lengths, and maturity data collected by size category, ≥25 cm TL. Ages were determined for 53 of these specimens.

		Number of samples	
Year	Sample type	Northwest	Southeast
1992	A	107	218
	L	0	1,260
1993	A	98	162
	O	256	512
1994	A	133	158

Age at maturity Analysis of female age at maturity was treated in the same manner as with length at maturity, substituting age (A) in years for length (L). Owing to smaller sample sizes (Table 2), data for years 1992–94 were pooled.

Results

Length at age

The general effects of area and bottom depth on length at age were highly significant for both males and females (Table 3). Mean length-at-age plots for both male and female yellowfin sole indicated greater sizes at age in the northwest than in the southeast area of the eastern Bering Sea shelf (Fig. 2). Male and female yellowfin sole were on average 1.22 and 1.02 cm larger at age, respectively, in the northwest area than in the southeast area. Average length-at-age differences between areas (northwest-southeast) increased with increasing age to more than 2 cm for both males and females (Fig. 3). Total lengths were generally greater at age in deeper (≥50 m) waters than in shallow waters (<50 m) for males and females less than 8 and 9 years of age, respectively (Fig. 3).

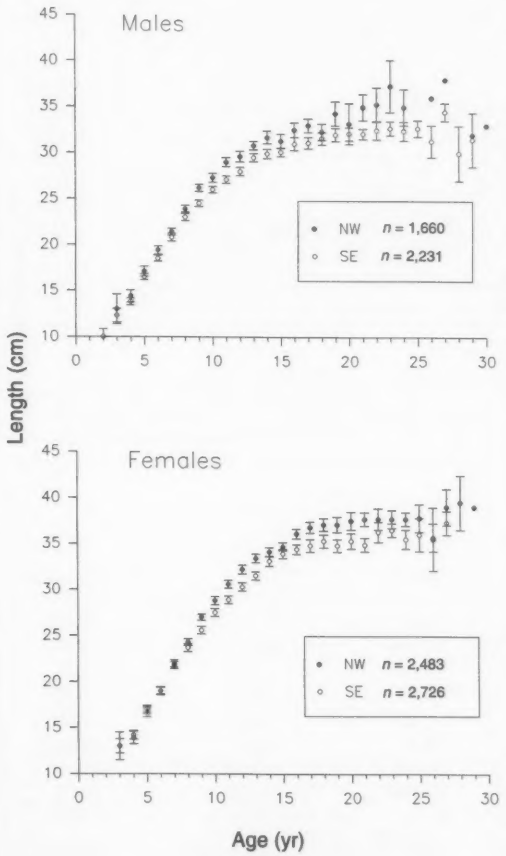

Figure 2

Comparison of male and female yellowfin sole mean length at age between northwest (NW) and southeast (SE) areas of the eastern Bering Sea (1982–94). Error bars indicate 95% confidence intervals.

Length at maturity

Female yellowfin sole lengths corresponding to 50% maturity (L_{50}) were greater in the northwest than in the southeast area, and L_{50} increased with increasing bottom depth (Fig. 4). Area accounted for a 2.3 cm female length-at-maturity difference (P=0.0001) in 1993 and a 0.91 cm difference (P=0.049) in 1994 (Fig. 4; Table 4). Female L_{50} increased with increasing bottom depth ($P \leq 0.013$), varying by as much as 4 cm between shallow and deeper waters (Fig. 4; Table 4). Annual variation (1992–94) in L_{50} appeared to be approximately 1 cm (Fig. 4).

Age at maturity

In contrast to length-at-maturity results, no significant age-at-maturity difference (P=0.080) was found for females between the two areas (Fig. 5; Table 5). A similar increase in female age at maturity, however, did occur with increasing bottom depth (P=0.0001), with age at 50% maturity increasing by 3 years from shallow to deeper waters (Fig. 5).

Discussion

Larger northwest fish lengths corresponding to a particular age or percent maturity, combined with no apparent age-at-maturity difference between areas, indicate faster yellowfin sole growth in the northwest area than in the southeast area. Bottom depth effects on length-at-age, length-at-maturity, and age-at-maturity estimates, however, were more the result of sampling uneven fish distributions, as discussed below.

Area effects

The length-at-age and length-at-maturity differences found between areas support the hypothesis that northwest and southeast complexes are functionally allopatric during the summer spawning period. Tag-

ging studies (Wakabayashi, 1989) indicated only limited movements of yellowfin sole between northwest and southeast areas as they migrate inshore, and Kashkina (1965) supported a two-stock concept, citing differences in egg-stage advancement and abundance between northern and southern regions of the eastern Bering Sea. Despite the lack of genetic evidence supporting the coexistence of two independent

Table 3

Factorial analysis of variance of male and female yellowfin sole length (cm) at age (years), by area (northwest and southeast) and bottom depth (<30, 30–49, and ≥50 m), pooled across years 1982–94. Note that nonsignificant ($P \geq 0.05$) main effects were retained because they were included in one or more significant interaction terms. SS = sum of squares.

Source	df	SS	Mean square	F-value	$P > F$
Males					
Age	29	68,007.6	2,345.1	360.0	0.0001
Area	1	295.5	295.5	45.4	0.0001
Depth	2	5.7	2.8	0.4	0.6457
Age × depth	49	1,906.9	38.9	6.0	0.0001
Age × area	24	160.4	6.7	1.0	0.4268
Area × depth	2	51.8	25.9	4.0	0.0188
Age × area × depth	34	366.9	10.8	1.7	0.0098
Error	3,749	24,421.2	6.5		
Females					
Age	29	152,746.0	5,267.1	667.9	0.0001
Area	1	408.1	408.1	51.8	0.0001
Depth	2	2.1	1.1	0.1	0.8738
Age × depth	49	3,219.2	65.7	8.3	0.0001
Age × area	24	411.2	17.1	2.2	0.0008
Area × depth	2	44.7	22.3	2.8	0.0589
Age × area × depth	44	593.1	13.5	1.7	0.0025
Error	5,057	39,880.8	7.9		

Table 4

Logistic regression coefficients for the equation $MAT=1/(1+e^{-(\mu+\beta \cdot L+\alpha \cdot \text{area}+\delta \cdot D+\Delta \cdot L \cdot D)})$ relating female yellowfin maturity status to fish length (L), binary variable area (northwest or southeast), and continuous variable depth (D). $L \times D$ denotes the interaction between length and depth. SE= standard error of the estimate. $L \times D$ coefficients were considered nonsignificant ($P \geq 0.05$) and were therefore removed from the model. Remaining coefficients and estimates result from model runs with $L \times D$ removed (underlined values).

			Coefficient			
Year	n	Variable	Symbol	Estimate	SE	$P >$ chi-square
1992[1]	1,478	Length	β	–0.82	0.047	0.0001
		Depth	δ	0.046	0.0048	0.0001
		L × D	Δ	0.0046	0.0024	0.057
		Intercept	μ	22.22	1.36	0.0001
1993	1,028	Length	β	–0.83	0.063	0.0001
		Area	α	2.29	0.30	0.0001
		Depth	δ	0.042	0.0078	0.0001
		L × D	Δ	–0.0065	0.0035	0.063
		Intercept	μ	21.70	1.81	0.0001
1994	291	Length	β	–0.69	0.085	0.0001
		Area	α	0.91	0.46	0.049
		Depth	δ	0.042	0.012	0.0004
		L × D	Δ	0.0046	0.0048	0.34
		Intercept	μ	17.92	2.52	0.0001

[1] Southeast area only. Small sample size in the northwest area (n=107) limited a comparison by area.

stocks (Grant et al., 1983), the persistence of area-based differences suggests that mixing of adults between northwest and southeast complexes may be minimal.

Growth-rate differences may be associated with geographic differences in yellowfin sole density or bottom temperature (or both). Yellowfin sole mean density (1982–94; author, unpubl. data), measured in catch per unit of effort (kg/hectare) during spring-summer, has been consistently higher in the southeast area (88.9 kg/ha) than in the northwest area (27.8 kg/ha). Spring-summer bottom temperatures have also been consistently higher in the southeast area than in the northwest area (Fig. 6). The higher yellowfin sole growth rate in the northwest areas is consistent with density-dependent hypotheses (Beverton and Holt, 1957; Cushing, 1975; Rijnsdorp, 1994) that suggest a negative correlation between fish growth and fish density. Reasons why fish growth appears faster in cooler northwest waters are less clear.

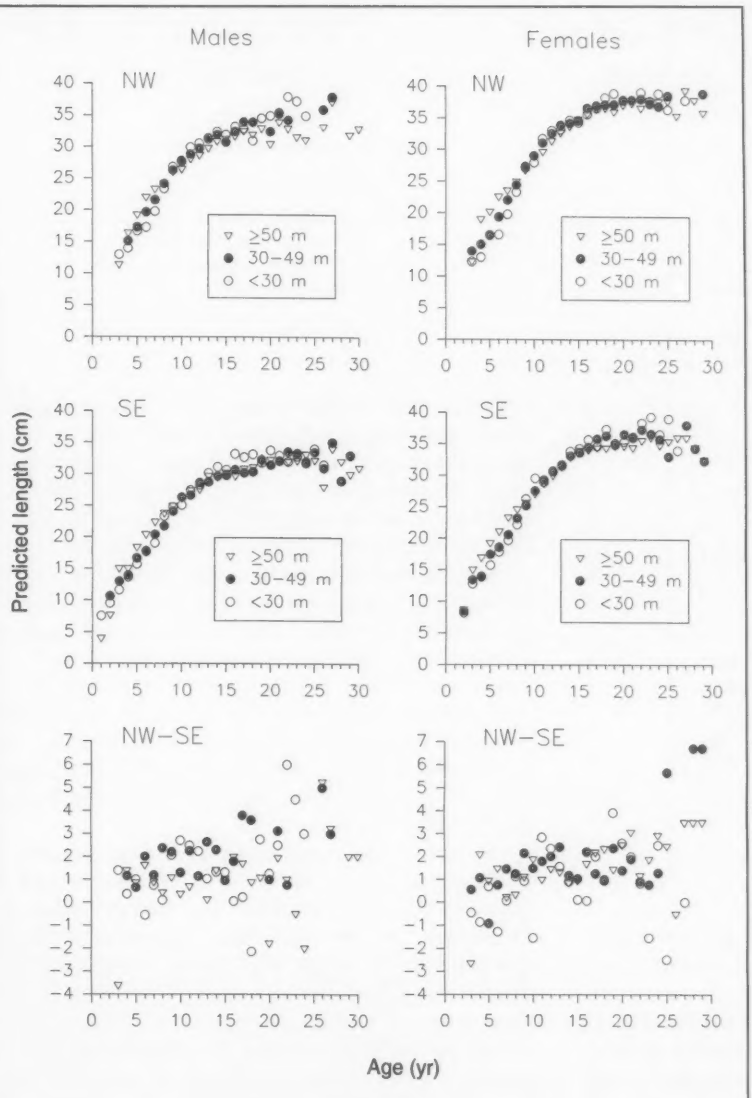

Figure 3

Factorial length at age model results comparing length at age of male and female yellowfin sole across bottom depths (m) in both northwest (NW) and southeast (SE) areas of the eastern Bering Sea. Age × Area × Depth interactions are included with data pooled across 1982–94 age-length collections.

Age-composition data used in stock assessments for yellowfin sole in the eastern Bering Sea (Wakabayashi et al., 1985) have been based upon age-length keys generated from annual age-length collections (Armistead and Nichol, 1993). Although independent age (otolith) collections have been made for the southeast and northwest areas, age-length keys are currently pooled across areas. The resulting estimates of growth are considered accurate because Alaska Fisheries Science Center age-structure collections have been spread fairly evenly between areas. However, given the spatial patterns described here, separate northwest-southeast age-length keys might improve the precision of these estimates.

Implications of depth-related sampling biases

Immature yellowfin sole, like many other fish species (Hunter et al., 1990; Macpherson and Duarte, 1991; Jacobson and Hunter, 1993), undergo an ontogenetic migration, distributing themselves along a size-depth continuum where smaller individuals inhabit shallow waters and larger individuals inhabit deeper waters. A single cohort can also distribute itself along this size-depth continuum. In doing so, faster-growing individuals can be found at

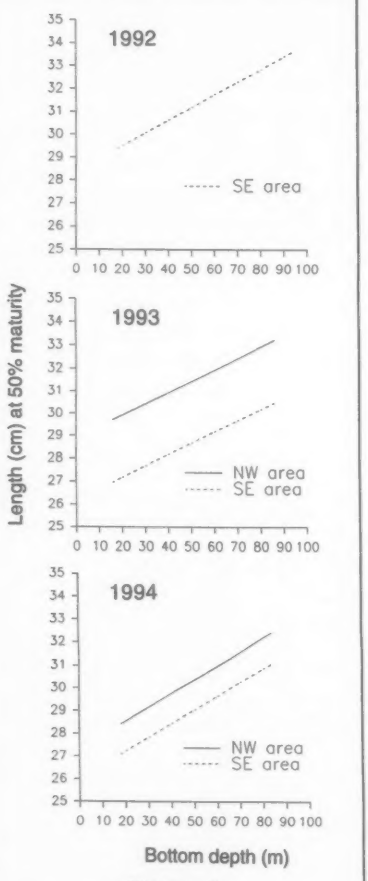

Figure 4

Female yellowfin sole length at 50% maturity, with respect to bottom depth (D) and geographic area (NW and SE) for years 1992–94. Curves illustrate predicted values of fish length (L) from the logistic equation: $MAT = 1/(1+e^{-(\mu+\beta \cdot L+\alpha \cdot area+\delta \cdot D)})$, where the proportion mature (MAT) is 0.5.

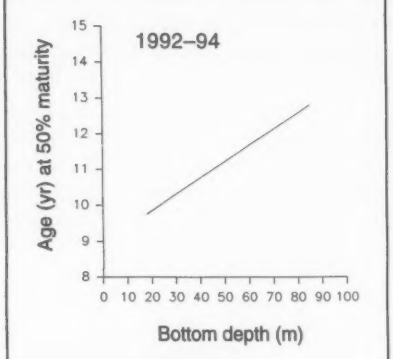

Figure 5

Female yellowfin sole age at 50% maturity, with respect to bottom depth (D), pooled among years 1992–94. Curves illustrate predicted values of fish age (A) from the logistic equation: $MAT = 1/(1+e^{-(\mu+\beta \cdot A+\delta \cdot D)})$, where the proportion mature (MAT) is 0.5.

deeper depths than can slower-growing individuals. Length-at-age estimates for immature yellowfin sole (<8 years of age) from shallower waters, therefore, are biased low in comparison with those from deeper waters (Fig. 3). The cessation of this depth effect with increasing bottom depth after 8 years of age (Fig. 3) may indicate the approximate age at which immature yellowfin sole leave the size-depth continuum and become migratory (i.e. to shelf-slope waters in winter and back to nearshore waters in spring-summer). The timing of first maturity may very well coincide with the initiation of a "spawning" migration.

The increase in female length and age at maturity with increasing bottom depth was largely due to the variation of immature female length distributions as depth increased. In spring-summer, when yellowfin sole have migrated nearshore for spawning, distributions of mature migratory individuals and immature "ontogenetically driven" individuals overlap. The differences between these two migration patterns act to separate immature and mature fish of similar lengths (and ages) along a bottom depth gradient. Immature females were distributed unevenly by size across depth; larger sizes (25–32 cm TL) were more common at deeper depths (Fig. 7). In contrast, mature females were distributed similarly by length between shallow (<30 m) and deeper (≥30 m) bottom depths (Fig. 7).

The absence of these larger immature females in shallow water resulted in lower values of length at maturity for shallow waters and higher values for deeper waters (Fig. 7). Trippel and Harvey (1991) demonstrated how age at maturity of white suckers (*Catostomus commersoni*) could be affected if year classes falling within the progression from immaturity to maturity are missing. Missing length classes, similarly, affect estimates of length at maturity. Sampling of female yellowfin sole in shallow waters (<30 m) misses critical length and age classes of immature individuals on the verge of maturity.

Length or age at maturity, as with growth relations, are often measured simultaneously across multiple cohorts and are therefore approximations

Table 5

Logistic regression coefficients for the equation $MAT=1/(1+e^{-(\mu+\beta\cdot A+\alpha\cdot area+\delta\cdot D+\Delta\cdot A\cdot D)})$ relating female yellowfin maturity status to fish age (A), binary variable area (northwest or southeast), and continuous variable depth (D). $A \times D$ denotes the interaction between age and depth. SE= standard error of the estimate. $A \times D$ and age coefficients were considered nonsignificant ($P \geq 0.05$) and were therefore removed from the model. Remaining coefficients and estimates result from model runs with $A \times D$ and age removed (underlined values).

			Coefficient			
Year[1]	n	Variable	Symbol	Estimate	SE	$P >$ chi-square
1992–94	929	Age	β	–0.70	0.048	0.0001
		Area	α	0.38	0.21	0.080
		Depth	δ	0.032	0.0056	0.0001
		A × D	Δ	–0.0040	0.0021	0.054
		Intercept	μ	6.25	0.50	0.0001

[1] Data were pooled among years 1992–94.

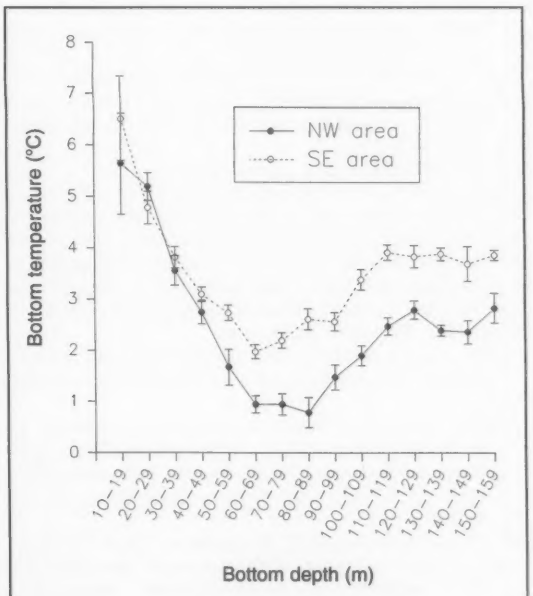

Figure 6

Mean bottom temperatures of the southeast and northwest areas of the eastern Bering Sea shelf, averaged across years 1982–94 at 10 m bottom depth intervals. Error bars indicate 95% confidence intervals.

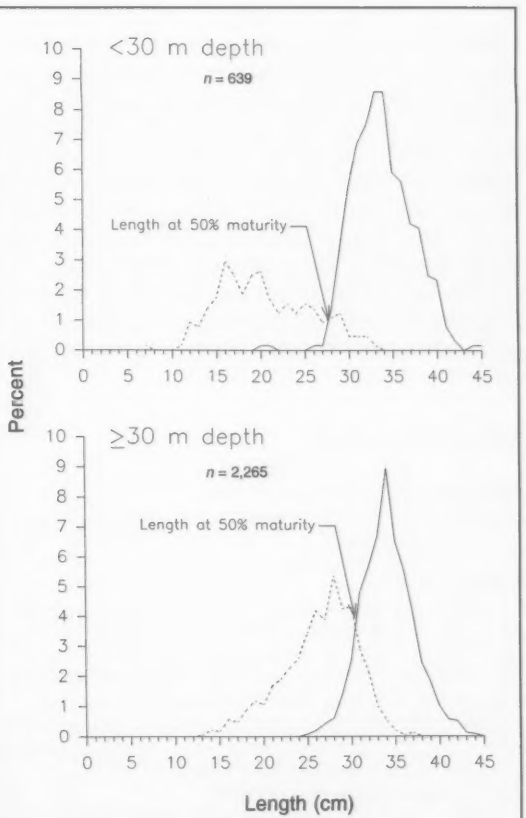

Figure 7

Length distribution of mature (solid line) and immature (dashed line) female yellowfin sole at bottom depths less than 30 m and greater than 30 m, 1992–94 data combined.

of individual growth and length or age at maturity. This estimation assumes that there is no between year-class growth or maturity differences with respect to a given age and that samples within each age class are random with respect to the population

(Ricker, 1975). Obtaining representative samples from each age class becomes difficult when fish size and age vary with bottom depth. Considering also that during spring-summer, yellowfin sole abundance increases with decreasing bottom depth (Nichol, 1995), population estimates of yellowfin sole growth and length or age at maturity should be weighted accordingly. Because groundfish assessment surveys in the eastern Bering Sea do not cover the shallowest areas of yellowfin sole distribution during spring-summer, current estimates of yellowfin sole growth, as well as size and age at maturity, are inherently biased high. Given that most demersal fish distribute themselves along a size-depth continuum (Macpherson and Duarte, 1991), the potential for similar depth-related sampling biases in other demersal fish species appears probable.

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Habitat models for juvenile pleuronectids around Kodiak Island, Alaska*

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Abstract.—Juveniles of four species of pleuronectid flatfishes were abundant in bays and nearshore areas around Kodiak Island, Alaska, during August 1991 and 1992. The four most abundant species of juvenile (age-0 or age-1) flatfishes were rock sole (*Pleuronectes bilineatus*), flathead sole (*Hippoglossoides elassodon*), Pacific halibut (*Hippoglossus stenolepis*), and yellowfin sole (*Pleuronectes asper*). These species appeared to share nursery areas; however, physical characteristics of the nursery areas occupied by each species limited the amount of true overlap among species. Tree-based regression of catch-per-unit-of-effort data on physical parameters was used to refine conceptual models of species distribution, which were originally based only on 1991 data.

Threshold values of the physical parameters were specified that best discriminated among stations with different abundances. Highest abundances of age-0 rock sole were found on sand or muddy sand at temperatures greater than 8.7°C, as well as on other mixed sand stations less than 28 m deep. Age-0 flathead sole were most abundant at temperatures less than 8.9°C and on mixed mud substrates. At warmer temperatures, abundances were high only if the depth was greater than 48 m, regardless of sediment type. Age-0 Pacific halibut were most abundant in depths less than 40 m at sites more than 2.9 km outside the mouths of bays. Inside bays, halibut were found in lower abundances in water over 9.0°C and on sediments containing both sand and mud. Age-1 yellowfin sole were always found in depths less than 28 m on mixed mud substrates. They were usually found within bays, with highest abundances at heads of large bays more than 32 km from the bay mouth. These four most abundant flatfishes therefore appeared to partition the available habitat in ways that minimized resource competition.

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In the Gulf of Alaska, there are directed fisheries for deep-water and shallow-water complexes of fishes. The deep-water complex is made up of rex sole (*Errex zachirus*), Dover sole (*Microstomus pacificus*), Greenland halibut (*Reinhardtius hippoglossoides*), arrowtooth flounder (*Atherestes stomias*), and rockfishes (*Sebastes* spp.). The shallow water complex incorporates all other flatfishes found in the area, including rock sole (*Pleuronectes bilineatus*), flathead sole (*Hippoglossoides elassodon*), yellowfin sole (*Pleuronectes asper*), English sole (*Pleuronectes vetulus*), starry flounder (*Platichthys stellatus*), Alaska plaice (*Pleuronectes quadrituberculatus*), butter sole (*Pleuronectes isolepis*), and sand sole (*Psettichthys melanostictus*), in addition to Pacific cod (*Gadus macrocephalus*) and walleye pollock (*Theragra chalcogramma*). The groundfish harvest from the Gulf of Alaska has been over 190,000 metric tons (t) annually from 1990 through 1995, for a total of 1,320,000 t, not including Pacific halibut (*Hippoglossus stenolepis*) or discards. Of that, in 1995, 716,000 t were landed in Kodiak, Alaska, for a value of $34 million (NMFS, Fisheries Management Div., P.O. Box 21668, Juneau, AK 99802-1668). When Pacific halibut, a species regulated separately from other groundfishes, is included, the total landed at Kodiak in 1995 was 75,000 t at $49 million. Although rockfishes (Carlson and Straty, 1981; Krieger, 1992, 1993), cod (Wespestad et al., 1986; Dunn and Matarese, 1987), and pollock (Janusz, 1986; Dunn and Matarese, 1987; Kendall et al., 1994; Müter and Norcross, 1994; Swartzman et al., 1994) have been studied in the Gulf of Alaska, very little is known about flatfishes (Parker, 1989; Moles and Norcross, 1995). The large abundance and value of these commercially important flatfishes and lack of knowledge of their early life history led us to investigate distribution of juvenile flatfishes around Kodiak Island.

In general, recently metamorphosed flatfishes recruit to shallow, nearshore nursery areas with fine-grained sediments (Edwards and Steele, 1968; Gibson, 1973; Toole, 1980; Hogue and Carey, 1982; de Ben et al., 1990). Intertidal zones, estuaries, and shallow protected bays are nursery areas for flatfishes in the continental United States (Krygier and Pearcy, 1986; Allen, 1988; Rogers et al., 1988; Wyanski, 1990), Canada (Tyler, 1971), Europe

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(McIntyre and Eleftheriou, 1968; Gibson, 1973; Lockwood, 1974; Poxton et al., 1982; Poxton and Nasir, 1985; van der Veer and Bergman, 1986), and Japan (Tanaka et al., 1989). Abundance and size distributions have been related to water depth (Edwards and Steele, 1968; McIntyre and Eleftheriou, 1968; Lockwood, 1974; Riley et al., 1981; Poxton et al., 1982; Wyanski, 1990), sediment size (Poxton et al., 1982; Poxton and Nasir, 1985; Wyanski, 1990; Jager et al., 1993; Keefe and Able, 1994; Moles and Norcross, 1995), and food availability (McIntyre and Eleftheriou, 1968; Allen, 1988; Jager et al., 1993). The generally accepted rationale for juvenile recruitment to shallow, fine-grained nursery areas includes escape from predation, increased cover and food availability, and decreased intraspecific food competition (Toole, 1980; de Ben et al., 1990; Minami and Tanaka, 1992). The examination of diet diversity among a subset of the fishes in the present study showed a reduction of both interspecific and intraspecific dietary overlap when flatfishes coexisted in large abundances (Holladay and Norcross, 1995).

The coastline of Kodiak Island, Alaska, encompasses a variety of habitats from shallow, fine-grained tidal flats to deep and rocky areas. Kodiak Island is mountainous and cut by many fjords and open bays with shallow waters (<10 m) usually within 0.5 km of the beach. The tidal range is 3 to 4 m. The region is characterized by deep bays, rough bottom topography, strong currents, and bottom characteristics that change rapidly over relatively short distances. Around Kodiak Island, juvenile flatfishes occupy fine-grained sediments in bays and nearshore waters, as do flatfishes in other locations, but waters less than 10 m in depth are only a minor component of the area that is used (Norcross et al., 1995).

A nursery may be partitioned into areas dominated by individual species or intraspecific age groups (Edwards and Steele, 1968; Zhang, 1988; Harris and Hartt[1]; Smith et al.[2]). Habitats occupied by juvenile rock sole, flathead sole, Pacific halibut, and yellowfin sole collected on the east and south sides of Kodiak Island in August 1991 can be differentiated on the basis of depth, substrate, and within-bay distribution (Norcross et al., 1995).

We used linear discriminant functions to identify tentively the habitat characteristics of juvenile flatfishes with data collected in August 1991 along east and south Kodiak Island (Norcross et al., 1995). In this study, we repeated the linear discriminant function analysis with combined 1991–92 data to include observations from a much wider geographic area around the entire island of Kodiak collected in August 1992. We refined our previous habitat models by using tree-based regression methods (Venables and Ripley, 1994) on catch-per-unit-of-effort data.

Materials and methods

Sample collections

Two cruises were conducted in the nearshore waters of Kodiak Island, Alaska, during August 1992 (Fig. 1). These cruises were similar to, but covered more area than, two cruises conducted in August 1991 (Norcross et al., 1995; Norcross et al.[3]). Cruise KI9201 consisted of collections taken with a 7.3-m skiff from Kalsin, Middle, and Womens Bays near the town of Kodiak during 9–14 August 1992. Because these bays were sampled with a skiff, extremely shallow collections could be made. Collections ranged in depth from 1 to 60 m. Ten stations were occupied in Kalsin Bay, six stations in Middle Bay, and five stations were occupied within Womens Bay. Kalsin and Middle Bays were also sampled during August 1991.

Immediately following the sampling from the skiff, a counterclockwise circuit of Kodiak Island was completed aboard a 24.7-m chartered trawling vessel (FV *Big Valley*, cruise KI9202). Collections during KI9202 were made from 16 to 29 August 1992 and ranged in depth from 5 to 180 m. Areas sampled in 1992, but not sampled in 1991, included 52 stations in bays on the north and west sides of the island. Collections were also made at 41 stations off south Kodiak, Sitkalidak Strait, and in Ugak Bay, which were sampled during August 1991.

Sampling gears, vessels, and vessel operators were the same in both 1991 and 1992 (Norcross et al., 1995; Norcross et al.[3]; Norcross et al.[4]). At each station one sediment sample was collected with a 0.06-m^3 Ponar grab for analysis of grain size, and a portable conductivity, temperature, and depth (CTD) profiler was deployed to measure temperature and salinity. Fishes

[1] Harris, C. K., and A. C. Hartt. 1977. Assessment of pelagic and nearshore fish in three bays on the east and south coasts of Kodiak Island, Alaska: final report. *In* Volume 1: Environmental assessment of the Alaskan continental shelf, p. 483–688. U.S. Dep. Commer., and U.S. Dep. Interior Quarterly Reports of Principal Investigators, Anchorage, AK.

[2] Smith, R. L., A. C. Paulson, and J. R. Rose. 1976. Food and feeding relationships in the benthic and demersal fishes of the Gulf of Alaska and Bering Sea. *In* Volume 7: Environmental assessment of the Alaskan continental shelf, p. 471–508. Annual Report RU 0284.7. U.S. Dep. Commer. and U.S. Dep. Interior Environmental Research Laboratories, Boulder, CO.

[3] Norcross, B. L., B. A. Holladay, and M. Frandsen. 1993. Recruitment of juvenile flatfish in Alaska, phase 1. Final Contract Report, NOAA NA-16FD0216-01, 504 p.

[4] Norcross, B. L., B. A. Holladay, F.-J. Müter, and M. Frandsen. 1994. Recruitment of juvenile flatfish in Alaska, phase 2. Final Contract Report, NOAA NA-26FD0156-01, 653 p.

were collected on rising tides during daylight hours by using a modified 3.7-m plumb staff beam trawl with a double tickler chain (Gunderson and Ellis, 1986). Tows of 10-min duration were made at the approximate speed of 0.5–1.0 kn from both the skiff and the trawler.

Sample processing

Substrate type, water depth, bottom temperature, bottom salinity, and distance from the mouth of the nearest bay were evaluated for each station. Sediment samples were analyzed, as in 1991, by means

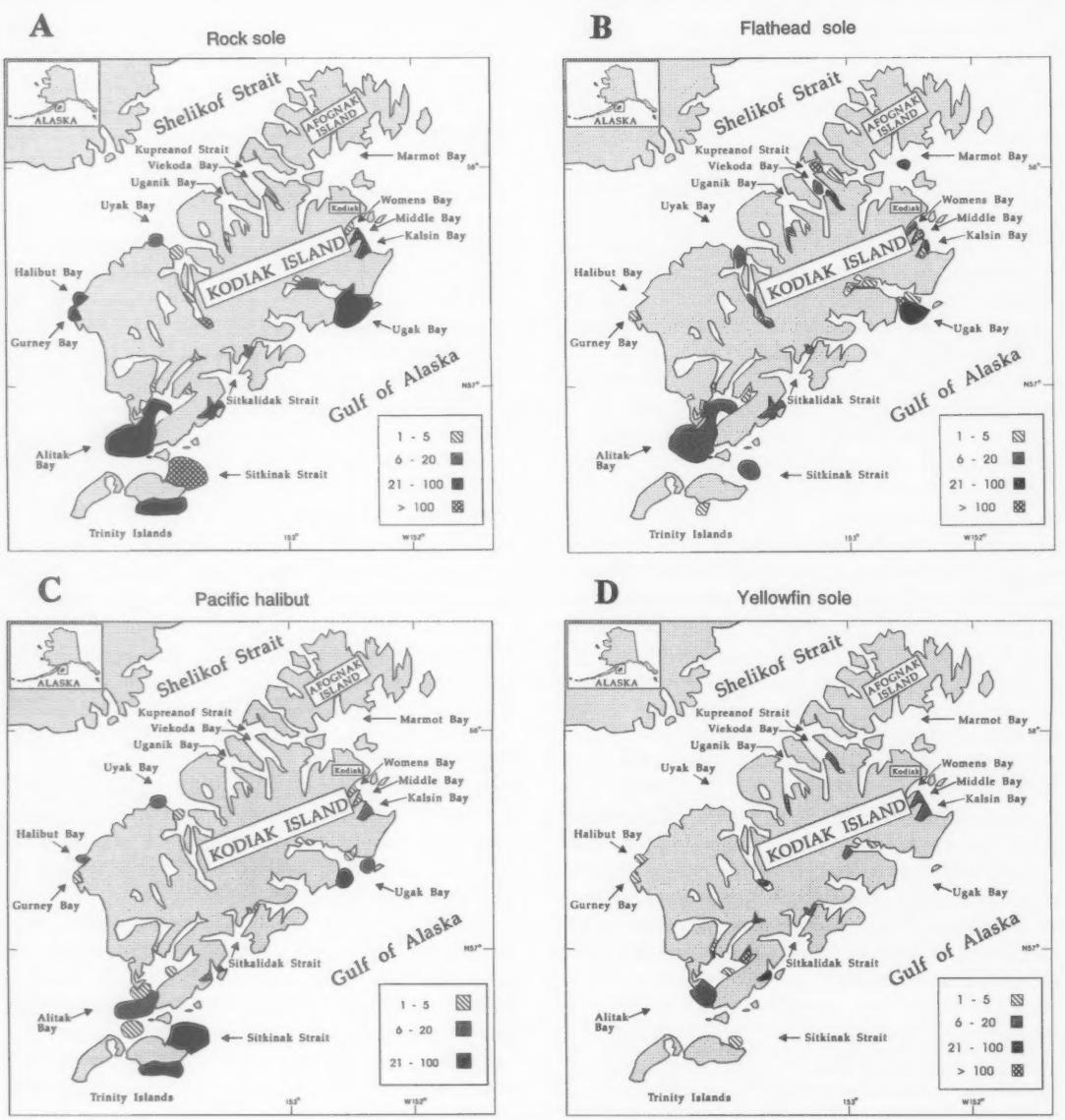

Figure 1

Distribution (CPUE) of (**A**) age-0 rock sole, (**B**) age-0 flathead sole, (**C**) age-0 Pacific halibut, and (**D**) age-1 yellowfin sole in August 1991 and 1992.

of a simplified sieve and pipette procedure to obtain the percents of gravel, sand, and mud (Norcross et al., 1995).

Distance from the mouth of the bay was used as a relative index of fish distribution with respect to station position within or outside the bay. Distance from each station to the nearest position at the mouth of a bay was calculated by drawing a line on a chart across the bay mouth between the two outermost capes. The shortest distance from the station to any position on this line was measured. Stations inside the mouth were designated as positive distances, and stations outside of bays were assigned negative distances. The narrowest point of Sitkinak Strait was considered the "mouth" of the bay; stations to the west of that point were considered within the bay and the exposed stations in the open ocean on the east side of Sitkinak Strait were considered outside the mouth.

Flatfishes were identified, and total length (mm) was measured in the field with a Limnoterra electronic, digital fish-measuring board. Ages of flatfishes captured in August 1992 were estimated with 1) length-frequency plots of fishes collected August 1992 (Norcross et al.[4]), 2) length-frequency plots (Norcross et al.[3]) and analysis of regional differences in total lengths (Norcross et al., 1995) of fish caught during August 1991, and 3) available literature (Southward, 1967; Best, 1974, 1977; Walters et al., 1985; Harris and Hartt[1]; Blackburn and Jackson[5]). Fish lengths were used to separate age classes of juvenile flatfishes. Catch per unit of effort (CPUE) based on a 10-min tow time was calculated for age-0 and age-1 individuals of each species. Habitat models were developed for the most abundant species and age-class combinations.

Statistical analyses

Linear discriminant function analysis of combined 1991–92 data included the broad range of conditions sampled around Kodiak Island. Canonical loadings of each variable and misclassification rates based on cross-validation were evaluated as outlined in Norcross et al. (1995) to test whether the same parameters had been selected as the best discriminators as those that had been selected solely on 1991 data. The magnitude of the canonical loading of each variable in the discriminant analysis is a measure of the importance of that variable in separating the stations with (presence) and without (absence) the fish species under consideration. The success of each combination of variables in assigning a new station to the presence or absence group can be evaluated by using misclassification rates from cross-validation.

The combined 1991 and 1992 data were further used to calculate Spearman's rank correlation (rho) between the abundance of each fish species and each physical parameter. The significance of rank correlations was evaluated at the 95% level. The nonparametric test with Spearman's rho was chosen because of non-normality of the CPUE data (even after transformation) and because of the high sensitivity of the parametric correlation coefficient (Pearson's r) to outliers. To maintain an overall confidence level of 95%, a Bonferroni-adjusted critical level of $\alpha = 0.025/28 = 0.001$ was used for the two-tailed test and for 28 comparisons (4 species × 7 variables).

To refine our previous habitat models, which were based primarily on presence or absence data (Norcross et al., 1995), we used regression trees to model CPUE as a function of habitat parameters. We used the same parameters as in the discriminant analysis, except instead of percentages of gravel, sand, and mud in the substrate, we used a categorical description of sediment type based on Folk (1980), i.e. sand (S), mud (M), gravel (G), and the modifiers of these substrates, such as sandy mud (sM), sandy gravelly mud (sgM), etc., 12 categories in all. This categorical classification avoided problems with high correlations among the three sediment variables. Both continuous and categorical predictor variables can easily be accommodated in regression trees.

The regression tree used the logarithm of CPUE (log(CPUE+1)) as the response variable and depth, distance from mouth of bay, bottom temperature, bottom salinity, and sediment type as predictor variables. A regression tree progressively splits stations on the basis of their values for one of the predictor variables until a leaf or terminal node is reached. Each leaf gives a predicted value of the response variable for the stations assigned to the leaf. The fit of the model is measured by the deviance, which is defined as

$$D = \Sigma_i (y_i - \mu_{[i]})^2,$$

or the sum of the squared differences between y_i = log(CPUE+1) at each station i and $\mu_{[i]}$ = the mean for all stations i at a leaf. The deviance is defined for the entire tree, as well as for each leaf, and is the analogue of the sum of squares in regression models. Each successive partitioning of the data reduces the deviance. For noisy data, the regression tree may overfit the data, resulting in an overly complex tree

[5] Blackburn, J. E., and P. B. Jackson. 1982. Seasonal composition and abundance of juvenile and adult marine finfish and crab species in the nearshore zone of Kodiak Island's east side during April 1978 through March 1979. *In* Outer continental shelf environmental assessment program, p. 377–570. U.S. Dep. Commer., Final Reports of Principal Investigators 54.

(Venables and Ripley, 1994). Therefore the initial tree was pruned to an optimum number of terminal nodes as determined by cross-validation.

Cross-validation as implemented in S-Plus (Venables and Ripley, 1994) uses 90% of the data as a training set to grow the tree and test it on the remaining 10%. This procedure is repeated 10 times with nonoverlapping test sets. Predictions on the test set are done for the initial tree as well as for trees pruned to smaller sizes. The resulting deviances are computed and plotted against tree size. Deviances typically are minimized at an intermediate tree size. We chose as optimum tree size the largest size before a marked increase in deviance occurred.

Results

Rock sole was the most abundant flatfish captured in our 1992 sampling (67% of flatfish), as in 1991 (51% of flatfish). In 1992, a total of 4,625 age-0 rock sole (17–60 mm TL) were collected across almost all locations, with the highest CPUE in the Sitkinak Strait region (Fig. 1A). Age-0 rock sole were found mainly near the mouths of bays ± 8–10 km, except for a single large catch at the head of Uyak Bay. Age-0 rock sole were somewhat more abundant with increasing depth between 0 and 30 m, and were collected in high numbers to 70 m, although they were also found deeper than 70 m. Age-0 rock sole were collected in large numbers between 7.5°C and 9.5°C and were most often found at salinities of 32.5–33.0 psu (Norcross et al.[4]). Rock sole were predominantly distributed on sand and mixed sand substrates. Although found at almost all combinations of depth and sand, rock sole were somewhat more concentrated in shallow, sandy locations (Fig. 2A). Spearman's rank correlation coefficients (Table 1) indicated that rock sole abundance was positively correlated with percent sand in the substrate and negatively correlated with depth, distance from mouth of bay, gravel, and mud. Rank correlation was highest with percent sand in the substrate.

Flathead sole increased from 12% of the 1991 catch to 18% of the 1992 catch. We captured 1,079 age-0 flathead sole (23–52 mm TL) during 1992. The distribution of flathead sole was more restricted than that for rock sole. Age-0 flathead sole were found almost everywhere around the island but were found in reduced numbers in Southeast Kodiak (Fig. 1B).

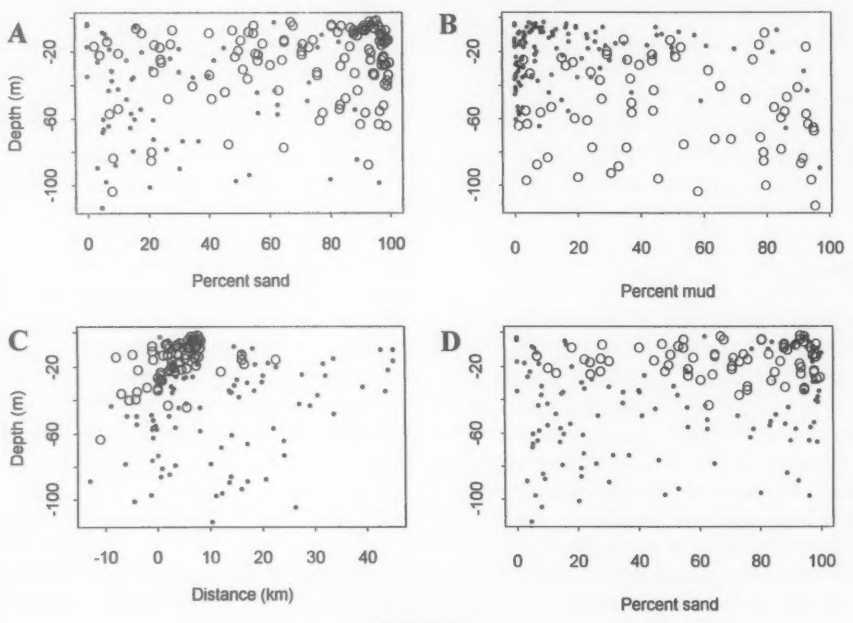

Figure 2

Presence (circles) and absence (dots) of (**A**) age-0 rock sole, (**B**) age-0 flathead sole, (**C**) age-0 Pacific halibut, and (**D**) age-1 yellowfin sole plotted against the two "best" discriminator variables. The depth axis is plotted with a 120-m limit, and data points occurring between 120 and 180 m are plotted at 120 m.

They were concentrated mainly in central, deep areas of bays at depths of 80–120 m, 6.0–9.0°C, 31.5–33.5 psu, on mud or mixed mud substrates (Norcross et al.[4]). High abundances of flathead sole were associated with deep stations, low temperatures, high salinities, low sand content, and high mud content; the highest rank correlations for flathead sole were obtained for depth and mud (Table 1). Flathead sole were predominantly collected in depths > 40 m, except on substrates with a high mud content (Fig. 2B).

Pacific halibut composed 5% of the catch in 1991 and 7% in 1992. During 1992, 627 age-0 halibut (22–84 mm TL) were found in exposed sites at all locations on the east and south sides of Kodiak Island (Fig. 1C). In northwestern Kodiak, halibut were collected only at the mouth of Uyak Bay. Age-0 halibut were found mainly at 10–70 m depth, 7.0–10.5°C, 32.0–33.0 psu, on mixed sand substrates, outside of or within 7 km of bay mouths (Norcross et al., 1995). Pacific halibut abundances were positively correlated with temperature and sand content and negatively correlated with depth, distance from mouth of bay, and mud content in the substrate. The highest rank correlations were with sand and mud (Table 1). Unlike rock sole, halibut were seldom found in water deeper than 50 m. Halibut juveniles, like rock sole, were concentrated most often in shallow waters with sandy substrate, near or outside mouths of bays (Fig. 2C).

Yellowfin sole was very abundant in 1991, composing 28% of captured flatfishes, but this species represented only 4% of the 1992 total catch. Unlike the other three species examined, in which age-0 fish predominated, age-1 yellowfin sole (41–105 mm TL) were analyzed in both 1991 and 1992 because of the small number (n=4) and size (15–20 mm TL) of age-0 yellowfin sole collected during the second year. During 1992, 268 age-1 yellowfin sole were collected at depths less than 40 m, mainly between 5 and 30 m. Age-1 yellowfin sole were found near the heads of bays, in warm (9.0–11.5°C) saline (31.0–33.5 psu) water (Norcross et al., 1995). They were collected on sandy mud, gravelly muddy sand, and muddy sand. Unlike rock sole and halibut, yellowfin sole were collected in the inner reaches of bays around Kodiak Island (Fig. 1D). The only significant correlation between yellowfin sole abundance and an environmental variable was a negative rank correlation with depth (Table 1). Yellowfin sole were never found deeper than 50 m and were always on mixed substrate, i.e. not predominantly on one grain size (Fig. 2D).

Linear discriminant function analysis for the combined 1991–92 data resulted in depth having the highest correlation with discriminant scores (canonical loadings) for flathead sole and yellowfin sole (Table 2). Sand was most highly correlated with the discriminant scores for rock sole and Pacific halibut. For all species, except flathead sole, the three highest canonical loadings were obtained for depth, temperature, and sand. In the case of flathead sole, mud was more highly correlated with the discriminant score than was sand.

Sand was clearly a good predictor for rock sole presence and was included in the habitat model for rock sole. Depth and temperature performed equally well in the discrimination owing to their high (negative) correlation. However, although rock sole abundance was significantly correlated with depth, the correlation with temperature was not significant. Therefore, sand and depth seemed to be the most important variables determining rock sole distribution (Fig. 2A).

The three best predictor variables for flathead sole were depth, gravel, and mud. Of these, depth and mud resulted in the lowest total error rates. Because

Table 1

Spearman's rank correlation coefficients between CPUE of four flatfish species and environmental parameters with 1991 and 1992 data combined. * indicates significance at an overall 5% confidence level.

Parameter	Rock sole	Flathead sole	Pacific halibut	Yellowfin sole
Depth	–0.258*	0.644*	–0.284*	–0.369*
Distance	–0.308*	–0.074	–0.314*	0.204
Temperature	0.193	–0.467*	0.346*	0.212
Salinity	–0.083	0.246*	–0.163	–0.192
Gravel	–0.240*	–0.219	–0.078	–0.168
Sand	0.583*	–0.219	0.449*	0.113
Mud	–0.310*	0.540*	–0.417*	0.188

Table 2

Canonical loadings from linear discriminant function analysis for combined 1991 and 1992 flatfish data.

Parameter	Rock sole	Flathead sole	Pacific halibut	Yellowfin sole
Depth	–0.557	–0.776	–0.620	–0.696
Distance	–0.379	–0.011	–0.501	0.234
Temperature	0.474	–0.597	0.647	0.545
Salinity	0.180	–0.225	–0.026	–0.005
Gravel	–0.453	0.321	–0.249	–0.377
Sand	0.783	0.220	0.655	0.406
Mud	–0.391	–0.624	–0.473	–0.099

these variables also had the largest rank correlations with abundance (Table 1), they were likely to be the most important parameters for flathead sole distribution. The error rates for predicting absence of flathead sole were consistently much lower than those for predicting presence.

Pacific halibut presence or absence could be most accurately predicted by using either depth or temperature with either distance or sand. Halibut abundance had a higher rank correlation with temperature than with depth and a higher correlation with sand than with distance from the mouth of the bay (Table 1). It is difficult to evaluate the relative importance of depth and temperature and of sand and distance owing to high correlations among these variables (Fig. 3). The depth-temperature factor explained most of the observed distribution. The error rates for predicting presence or absence changed significantly only if both depth and temperature were excluded. Error rates for stations where Pacific halibut were present were consistently much lower than those for stations where no halibut were found, thus this species appears to be strongly associated with specific habitat characteristics.

The three best predictors for yellowfin sole were depth and gravel combined with either sand or temperature. Of these, depth and gravel resulted in the lowest total error rates. Only depth was significantly correlated with yellowfin sole abundance (Table 1). The sediment parameters added very little information because yellowfin sole occurred over a wide range of substrate types. Error rates for stations where yellowfin sole were present were much lower than those for stations where this species was absent, reflecting the restricted depth range within which yellowfin sole were encountered. Presence and absence patterns for all four species are plotted against the two best discriminator variables (Fig. 2).

Regression trees were constructed by using CPUE for each species to refine our habitat models. The initial trees were allowed to grow, provided the number of stations in a node was five or greater. The resultant regression trees had sizes of 22 terminal nodes for rock sole, 16 for flathead sole, 19 for Pacific halibut, and 18 for yellowfin sole. The total deviances for the initial trees were 1.24, 0.57, 0.38, and 0.44 respectively, indicating that the model fitted for rock sole was much poorer than that for the other species and that the tree for Pacific halibut had the best fit.

The trees for all species seemed to overfit the data, as indicated by cross-validation. Plots of deviance against tree size (number of terminal nodes) for the four flatfish species indicated that deviance was usually at a minimum at very small tree sizes, consisting of only two or three nodes (Fig. 4). The deviance for each species tended to increase steeply at a tree size between 4 and 6 nodes, and we chose the largest size before a steep increase as optimum size for the tree. The initial tree was pruned to six terminal nodes for rock sole and halibut and to four terminal nodes for flathead sole and yellowfin sole.

The pruned regression tree for rock sole indicated that sediment, depth, and temperature were the best predictor variables for rock sole CPUE. The deviance of the pruned tree increased to 1.852 from 1.242 for the initial tree. This relatively poor fit may again be due to the widespread distribution of rock sole, a species that does not seem to be limited to any particular habitat type. Stations were first separated by sediment type into 89 stations on sand or muddy sand with a high mean CPUE (18 fish/10-min tow) and 80 stations on other sediment types that had a much lower mean CPUE (1.6 fish/10-min tow) (Fig. 5). The highest mean CPUE (25 fish/10-min tow) occurred at stations on sand or muddy sand which had a bottom temperature of more than 8.7°C. The colder stations on sand and muddy sand were separated into seven low salinity stations with low mean CPUE (0.58 fish/10-min tow) and 10 high salinity stations with medium to high CPUE (11 fish/10-min tow). Most stations on other sediment types, which included gravel, mud, gravelly mud, gravelly sand, gravelly muddy sand, gravelly sandy mud, muddy gravel, muddy sandy gravel, sandy gravel, and sandy mud, had low CPUE values except for a group in shallow water (<27.5 m) on gravelly muddy sand, sandy gravel, or sandy mud (13 fish/10-min tow). Thus, by combining results from the correlation analysis, presence and absence patterns, and regression trees, rock sole were found to be most common on sand or mixed sand substrates and most abundant in shallow and relatively warm water.

The regression tree for flathead sole indicated that temperature, sediment type, and depth were the best predictors of flathead sole abundance. The deviance of the pruned tree was 0.774 compared with 0.569 for the initial tree. Highest CPUE values tended to occur at stations where bottom temperature was less than 8.9°C (Fig. 6). At warmer stations, mean CPUE of flathead sole was very low (0.17 fish/10-min tow) if stations were less than 48 m deep, which was the case for the majority (n=109) of the stations. Mean CPUE at warm stations was higher, however, for the six stations located in water deeper than 48 m (4.6 fish/10-min tow). Stations with bottom temperatures below 8.9°C had a low flathead sole CPUE if the sediment was categorized as gravel, sand, muddy sandy gravel, or sandy gravel (1.6 fish/10-min tow). The CPUE was much higher on pure mud or mixed mud

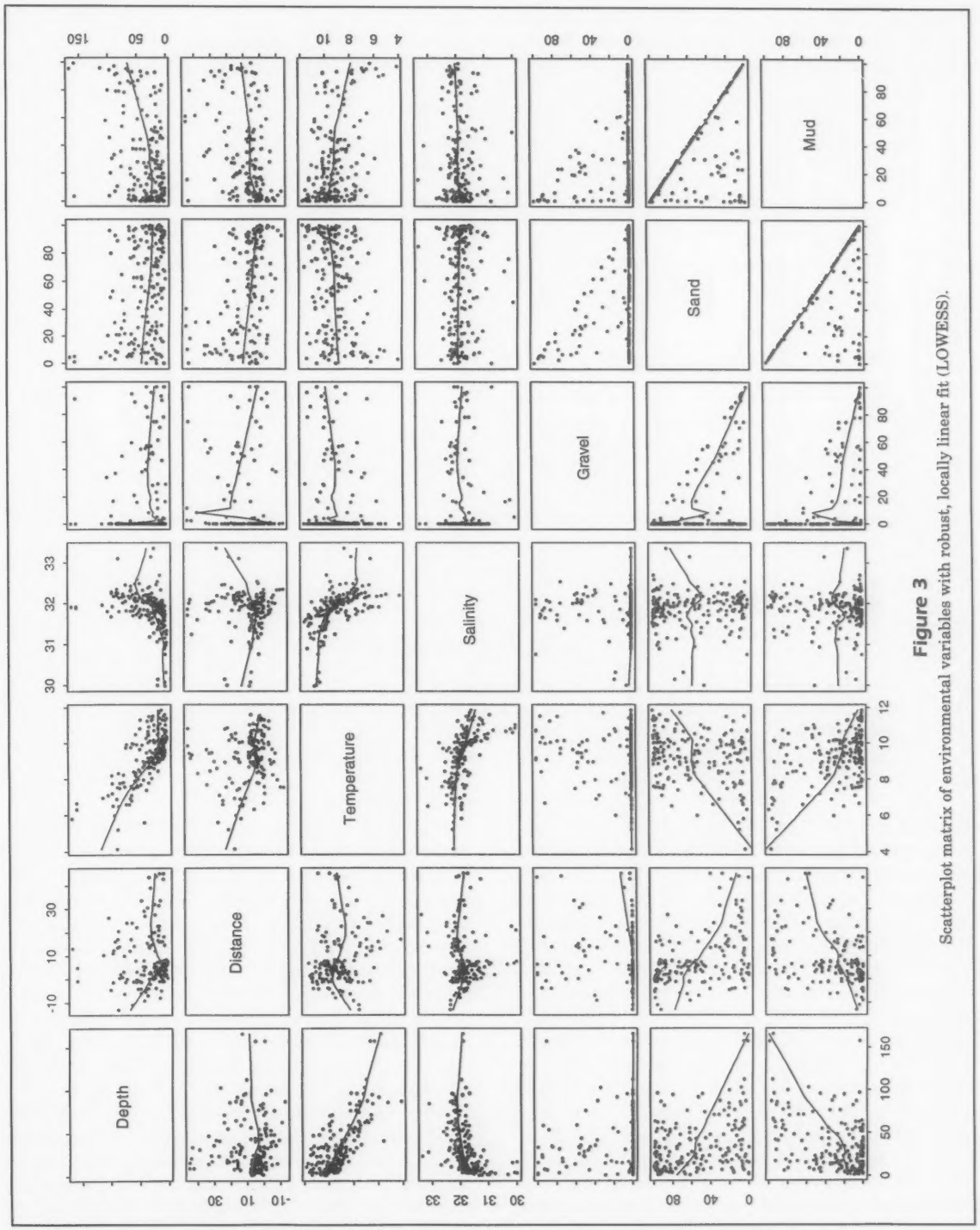

Figure 3
Scatterplot matrix of environmental variables with robust, locally linear fit (LOWESS).

sediments at low temperatures (8.0 fish/10-min tow). Thus, the highest CPUE values for flathead sole were on mixed mud sediments at stations with a bottom temperature of less than 8.9°C, as well as at warmer stations if they were deeper than 48 m. This suggests that temperature should be used in addition to

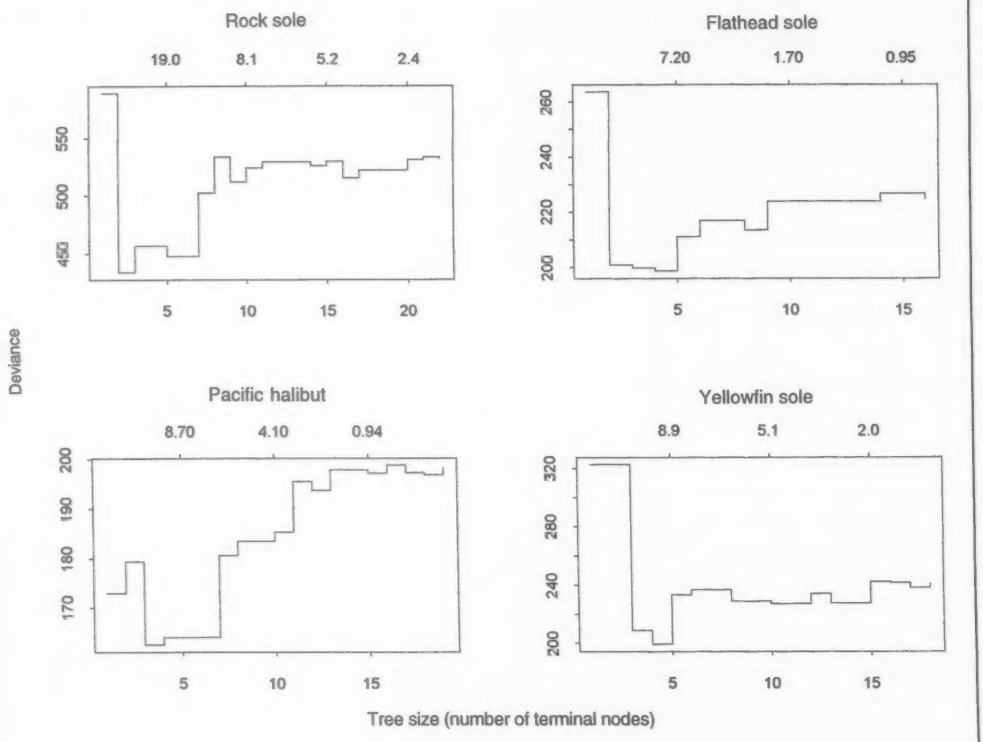

Figure 4

Cross-validation plots (deviance versus tree size) used to prune regression trees of catch per unit of effort on five habitat parameters. See text for further explanation.

sediment and depth selected by linear discriminant analysis as an important factor in determining the distribution of juvenile flathead sole.

Distance from the mouth of the bay and depth were the best predictors of halibut CPUE (Fig. 7) The deviance of the pruned tree was 0.587 compared with 0.384 for the initial tree. Highest CPUE values occurred at stations less than 40 m deep and more than 2.9 km outside the mouth of bays (10 fish/10-min tow). Very low abundances or no halibut were found at stations more than 7.9 km up the bay (0.13 fish/10-min tow). Intermediate CPUE values were found at 61 stations near the mouth of bays (–2.9 km to 7.9 km) which had high bottom temperature (>9.0°C) on sand or mixed sand substrates (2.9 fish/10-min tow). This confirmed our earlier finding that halibut tend to remain outside or near the mouth of bays in water less than 40 m deep on sandy substrates.

The most important variables used in predicting yellowfin sole abundance were depth, sediment, and distance. Deviance was increased from 0.442 for the initial tree to 0.895 for the pruned tree. The first split separated 100 stations less than 28 m deep from 69 stations deeper than 28 m (Fig. 8). The deeper stations had a very low mean CPUE (0.17 fish/10-min tow), and yellowfin sole were absent at 64 of the 69 stations that were deeper than 28 m. The shallow stations had a low mean CPUE (0.82 fish/10-min tow) if the substrate type was pure gravel, sand, or mud, or had mixed gravel sediment, whereas stations on mixed mud sediments had medium to high abundances of yellowfin sole (9.0 fish/10-min tow). The 53 shallow stations on mixed mud substrates were further split by distance, indicating that the highest CPUE values occurred near the heads of long bays. Thus yellowfin sole tended to be concentrated in very shallow locations on mixed mud sediments near the head of bays. This finding agreed with results of the linear discriminant function in its identification of depth and sediment as important factors, but further added distance from the bay mouth as a third important factor.

Discussion

Our results show that relations among flatfish distributions and habitats found within the geographic restrictions of eastern Kodiak Island in 1991 can be applied more broadly to other areas around Kodiak

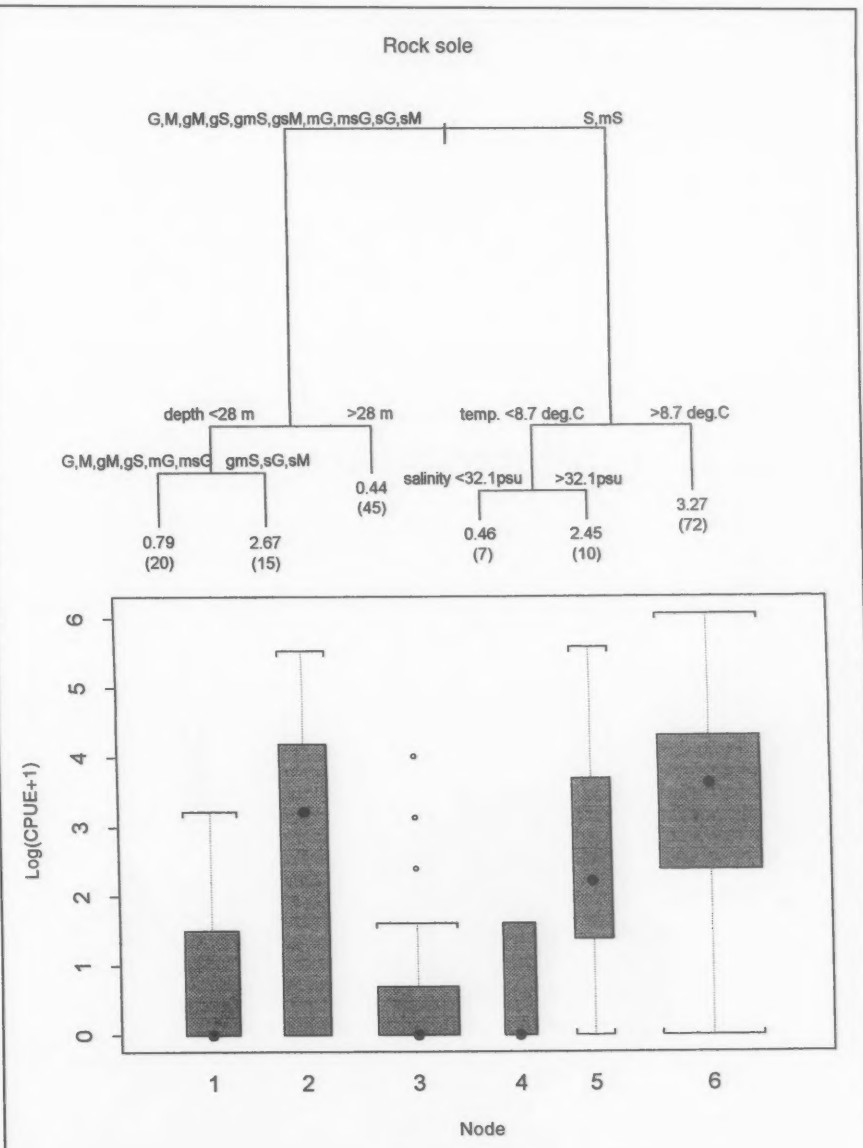

Figure 5

Pruned regression tree for age-0 rock sole with predictions of log(CPUE+1) at each terminal node, and number of stations in parentheses (top figure). Bottom figure contains box plots of log(CPUE+1) by terminal node. Each box plot summarizes distribution of CPUE at stations represented by the terminal node directly above it. Box plots indicate median (filled circle), interquartile range (box height), and outliers (open circles). Whiskers indicate upper quartile plus 1.5 times interquartile range and lower quartile minus 1.5 times interquartile range. Width of boxes is proportional to square root of number of stations at that node. See text for sediment abbreviations.

Island, i.e. to those areas sampled during 1992. Two groups of variables explain much of the observed distribution. These variables are substrate composition and a depth-temperature factor. The relative importance of depth and temperature or of gravel, sand, and mud is difficult to assess because each group is

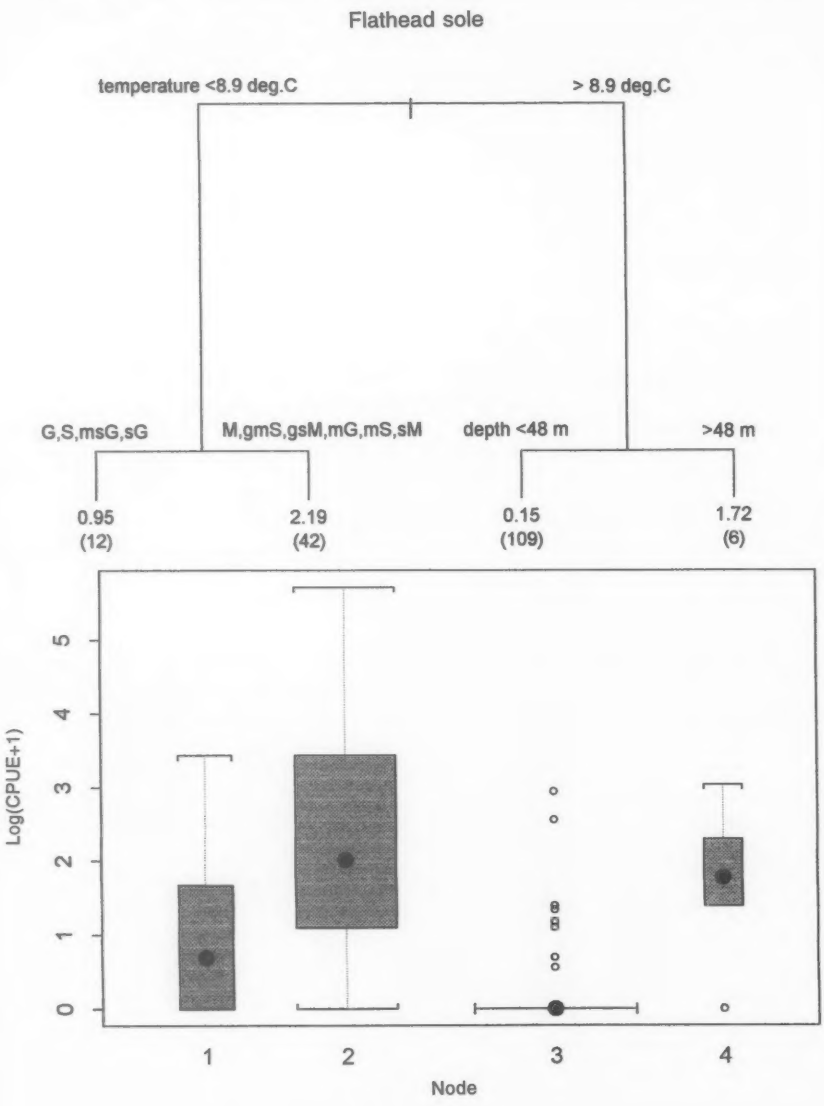

Figure 6

Pruned regression tree for age-0 flathead sole with predictions of log(CPUE+1) at each terminal node, and number of stations in parentheses (top figure). Bottom figure contains box plots of log(CPUE+1) by terminal node. Each box plot summarizes distribution of CPUE at stations represented by the terminal node directly above it. Box plots indicate median (filled circle), interquartile range (box height), and outliers (open circles). Whiskers indicate upper quartile plus 1.5 times interquartile range and lower quartile minus 1.5 times interquartile range. Width of boxes is proportional to square root of number of stations at that node. See text for sediment abbreviations.

highly intercorrelated (Norcross et al., 1995; Fig. 3).

These parameters have been linked to the habitat quality of juvenile flatfishes in many other locations (Gibson, 1994). Larvae of many flatfish species are known to settle either in shallow water (Edwards and Steele, 1968; Lockwood, 1974) or offshore water

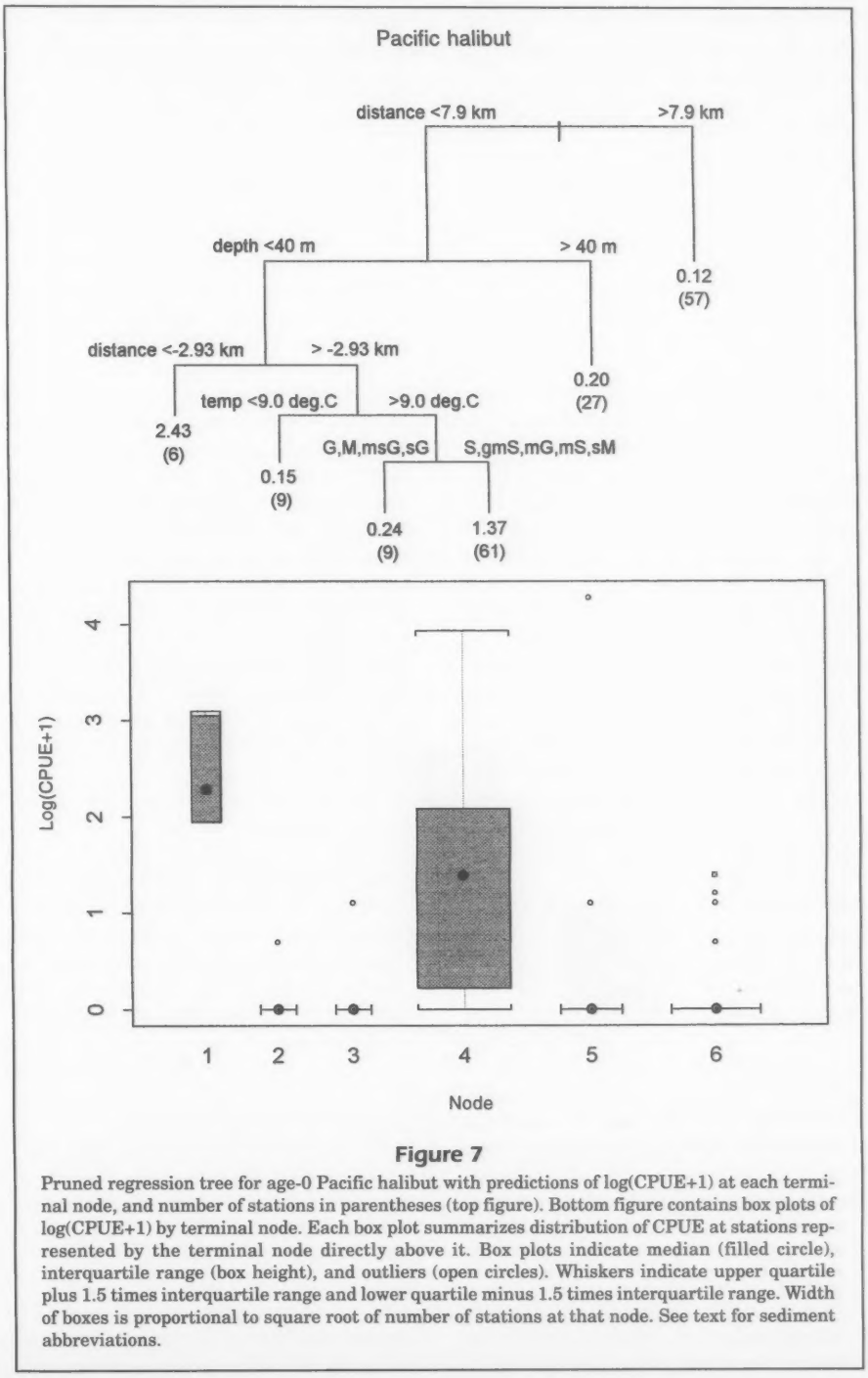

Figure 7

Pruned regression tree for age-0 Pacific halibut with predictions of log(CPUE+1) at each terminal node, and number of stations in parentheses (top figure). Bottom figure contains box plots of log(CPUE+1) by terminal node. Each box plot summarizes distribution of CPUE at stations represented by the terminal node directly above it. Box plots indicate median (filled circle), interquartile range (box height), and outliers (open circles). Whiskers indicate upper quartile plus 1.5 times interquartile range and lower quartile minus 1.5 times interquartile range. Width of boxes is proportional to square root of number of stations at that node. See text for sediment abbreviations.

and then to move into shallow water as age-0 juveniles (Gibson, 1973; Lockwood, 1974; Tanaka et al., 1989). In prior studies (Gibson, 1994) as well as this one, depth and its effect on water temperature may play an important part in determining distribution of juveniles. Water temperature affects growth and

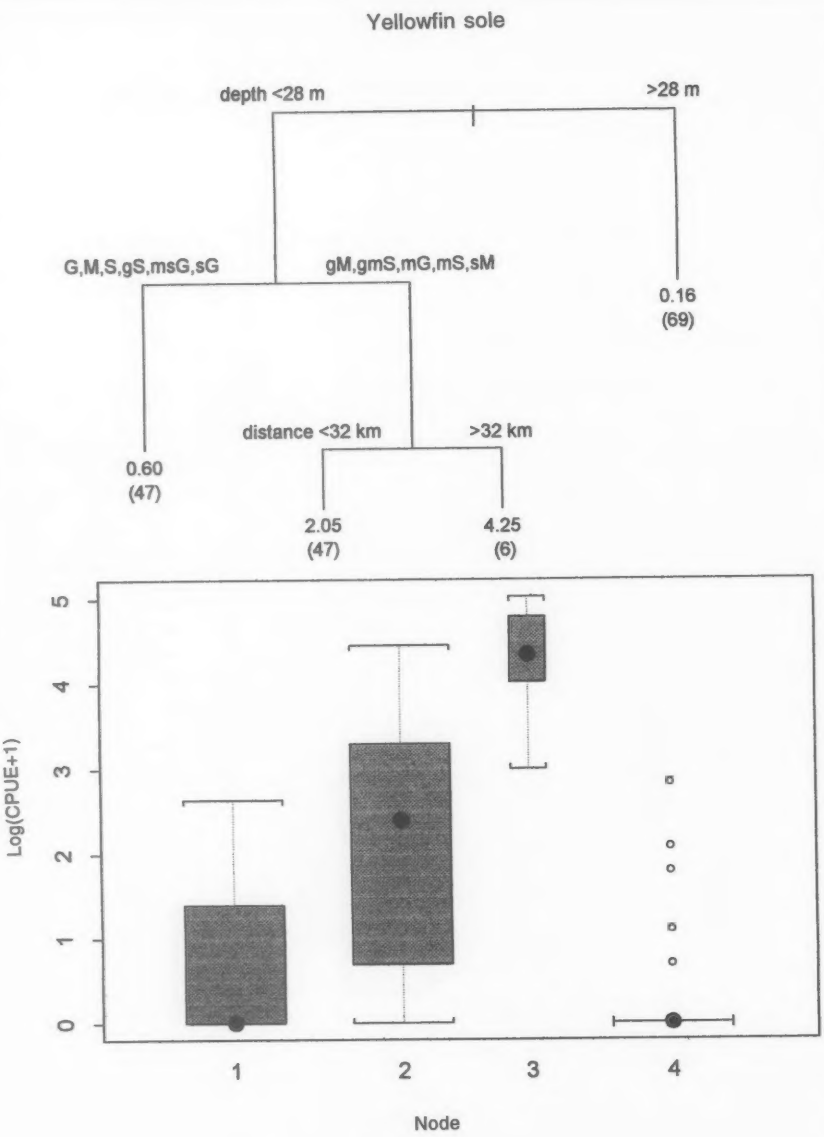

Figure 8

Pruned regression tree for age-1 yellowfin sole with predictions of log(CPUE+1) at each terminal node, and number of stations in parentheses (top figure). Bottom figure contains box plots of log(CPUE+1) by terminal node. Each box plot summarizes distribution of CPUE at stations represented by the terminal node directly above it. Box plots indicate median (filled circle), interquartile range (box height), and outliers (open circles). Whiskers indicate upper quartile plus 1.5 times interquartile range and lower quartile minus 1.5 times interquartile range. Width of boxes is proportional to square root of number of stations at that node. See text for sediment abbreviations.

feeding rates, and shallow, warm waters promote faster growth (Malloy and Targett, 1991; van der Veer et al., 1994).

Distribution of juvenile flatfishes has been linked to substrate type (Tanda, 1990; Kramer, 1991; Gibson and Robb, 1992). Juvenile flatfishes appear to avoid coarse sediments (Moles and Norcross, 1995) and choose fine-grained sediments (Rogers, 1992; Keefe and Able, 1994) which vary in size from mud (Wyanski, 1990; van der Veer et al., 1991) to sand (Jager et al., 1993). In laboratory tests, rock sole prefer sand and mixed sand substrates, halibut prefer a combination of mud and fine sand, and yellowfin sole prefer mud and mixed mud sediments (Moles and Norcross, 1995); these findings are in agreement with the classification and regression trees of our study. Choice of settlement location is affected by the ability of a fish to bury itself in the sediment (Gibson and Robb, 1992) as well as by the availability of prey in the substrate (Burke et al., 1991). When diets of juveniles of the four species were examined from the same collections in 1991 that were used in these models, it was found that epibenthic crustacean taxa composed most of the diets (Holladay and Norcross, 1995). Stomach contents were related to physical parameters of capture, including location, depth, and substrate. When distribution of juveniles overlapped, dietary overlap was sometimes reduced, in that one or more groups of flatfishes appeared to alter their feeding (Holladay and Norcross, 1995), i.e. preference for specific prey types did not appear to be a primary factor governing distribution of these species.

A discriminant analysis was employed in this study to test whether stations could be accurately classified into groups defined by the presence or absence of a given flatfish species. The classification based on the observed parameters resulted in relatively high error rates for all species; between one-sixth and one-third of the stations were incorrectly classified.

Although no discrimination method is able to predict perfectly the presence or absence of populations that have a gradation in abundance in marginal habitats, there are several possible reasons for the observed high error rates found in this study. For rock sole, halibut, and yellowfin sole, error rates for predicting presence were generally much lower than error rates for predicting absence. This finding may indicate that these species were mostly confined to relatively well-defined depth-substrate characteristics. The high misclassification rate for predicting absence of rock sole, halibut, and yellowfin sole suggests that many stations may offer suitable depth, temperature, and substrate conditions for these species but that the species are not collected there because their physical habitat preferences may be different. The situation is different for flathead sole; their presence is not as predictable as their absence. Flathead sole are generally absent from shallow areas with little mud, whereas they are usually, but not always, present in deep, muddy places.

The classification results suggest that although the seven environmental variables (%sand, %mud, %gravel, depth, temperature, salinity, and distance from bay mouth) used in our discriminant analysis do not account fully for observed flatfish distributions, they do provide a useful first step at defining juvenile flatfish habitat near Kodiak. The initial linear discriminant function models developed with the 1991 data (Norcross et al., 1995) are still applicable after incorporating 1992 data. Similar linear discriminant methods have been used to examine nursery grounds of *Solea solea* (Rogers, 1992).

Regression trees of CPUE for each species generally agree with the results of the linear discriminant analyses. They determine specific values of the physical parameters as related to the abundance of juvenile flatfishes and, as easily comprehensible diagrams, can be used to predict species abundance based on habitat parameters.

This detailed analysis, based on CPUE and incorporating both 1991 and 1992 data, does not disagree with the original models that we were testing (Norcross et al., 1995) but rather refines those models and incorporates actual abundances (CPUE) in the multivariate analysis. The previous models characterized nursery areas of age-0 rock sole, flathead sole, Pacific halibut, and age-1 yellowfin sole on the basis of correlations and discriminant analyses by using presence or absence for 1991 data. Depth and substrate were statistically significant variables previously, and a measure of distance in relation to mouth of the bay was included qualitatively for each species. Depth, temperature, sediment composition, and distance from bay mouth were all found to be important predictors of the abundance of juvenile pleuronectids with regression trees for the combined 1991 and 1992 data.

Additional factors influence the presence or absence of these flatfish species at any given site. Possible factors that were not included in this study are additional measures of location (such as position around the island or distance from shore), abundances of prey or predators, and a substrate or habitat parameter that would account for microhabitat features not reflected in sediment composition.

A location parameter may be a categorical variable that assigns each station to a well-defined geographical area. For example, we observed large differences in the abundance of halibut and rock sole

between the east and west sides of Kodiak Island and among different bays. These differences possibly reflect oceanographic conditions that lead to variable levels of recruitment into different nearshore areas around Kodiak Island. Habitat models incorporating geographical and oceanographic information may help to reveal these mechanisms but would require larger sample sizes than are presently available.

The abundance of prey (McIntyre and Eleftheriou, 1968; Minami, 1986; Allen, 1988) and predators (van der Veer et al., 1991; Seikai et al., 1993) may influence the distribution and abundance of flatfish species but cannot be quantified without extensive surveys. Incorporating prey or predator abundance into a general habitat model is therefore probably of little practical use in applying the model to other areas. Postmetamorphic flatfishes in southeastern Alaska (Sturdevant, 1987) and juvenile flatfishes near Kodiak (Holladay and Norcross, 1995) feed primarily on small meiofaunal, benthic, and epibenthic crustaceans, including mysids, amphipods, cumaceans, and copepods. The diets of flathead sole, Pacific halibut, yellowfin sole, and rock sole were different in different capture sites, when region, depth, and substrate were the parameters used for the sites. This finding suggests that these species are opportunistic and feed on the prey available in their locale, rather than that they are discriminating, determining locale on the basis of prey availability.

Additional information is desirable to describe the microhabitat at each station more precisely. During our sampling, we obtained qualitative descriptions of the benthic flora and fauna that were collected at each station and a very broad quantification of the dominant invertebrates that were caught together with the fishes. In the future, we will attempt to consolidate this information into a categorical "community descriptor" for each station. This "community descriptor" can then be used as an additional explanatory variable in future models.

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Daily growth increments in otoliths of juvenile weakfish, *Cynoscion regalis*: experimental assessment of changes in increment width with changes in feeding rate, growth rate, and condition factor

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Abstract.—Laboratory analyses were conducted on age-0 weakfish, *Cynoscion regalis*, to determine if deposition rate of otolith increments was daily and to examine the relation among otolith increment growth, daily feeding rate, specific growth rate, and condition factor. Tetracycline-marked juveniles (n=58) had a mean deposition rate of 0.98 (0.03 SE) increments/d. Feeding rate significantly affected increment width and was positively correlated with somatic growth rate and condition factor. Increment width response to changes in ration level was immediate, significant differences occurring between day 7 and 14. Mean increment width and specific growth rate were positively correlated (r=0.86). The continuation of otolith growth during periods of negative fish growth reflects the conservative nature of otolith growth and the lack of otolith resorption. An established relation between known growth rates of juvenile weakfish in the laboratory and otolith increment width will allow otolith increment widths to be applied to field samples. Such analyses could be used to examine closely factors affecting growth, survival, and recruitment.

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Growth rates in fishes can be determined by using scales, otoliths, modal analysis, RNA-DNA ratios, and assorted skeletal structures (Bagenal, 1978; Campana and Neilson, 1985; Summerfelt and Hall, 1987). Interpretations from these structures are based upon assumptions that increments within otoliths are added periodically and that the change in thickness of consecutive rings is proportional to fish length (Campana and Neilson, 1985). Otoliths have also proven to provide an accurate record of fish growth because there has been no evidence of resorption (Degens et al., 1969; Dunkelberger et al., 1980; Watabe et al., 1982; Mugiya, 1987), except under extreme physiological stress (Mugiya and Uchimura, 1989).

The periodicity of increment addition has been shown to be daily in larval and juvenile fishes (Campana and Neilson, 1982; Hettler, 1984; Schmitt, 1984; Tsukamoto, 1985; Wilson et al., 1987; Tzeng and Yu, 1989; Monaghan, 1993). Otolith microstructure has more recently been used to compare growth of different cohorts within a year class (Townsend and Graham, 1981; Warlen, 1982; Methot, 1983; Jones, 1985), examine life history transitions (Brothers and McFarland, 1981; Laroche et al., 1982; Powell, 1982; Miller and Storck, 1982; Victor, 1986; Thresher and Brothers, 1989), estimate mortality and survival (Crecco et al., 1983; Graham and Townsend, 1985; Neilson and Geen, 1986; Essig and Cole, 1986; Dalzell et al., 1987; Post and Prankevicius, 1987; Rice et al., 1987), and determine the effects of biotic and abiotic factors on microstructure

(Taubert and Coble, 1977; Tanaka et al., 1981; Campana and Neilson, 1982; Neilson and Geen, 1982, 1985; Neilson et al., 1985; Maillet and Checkley, 1991; Moksness, 1992).

Growth histories of fish are determined primarily through back calculation by using either a direct proportion or some nonlinear relation between otolith size and fish age (Maciena et al., 1987; Thorrold and Williams, 1989). Recent studies have used the relation between increment width (IW) and growth rate to show growth histories (Maillet and Checkley, 1990; Molony and Choat, 1990; Wright et al., 1990). Key assumptions for this use are that distance between increments is proportional to growth rate and that the increments are produced daily (Beamish and McFarland, 1983).

The objectives of this study were to validate the daily periodicity of otolith increments in juvenile weakfish, *Cynoscion regalis*, and to describe the relation between otolith IW and changes in feeding rate, specific growth rate, and condition factor.

Materials and methods

Experiment 1 (daily otolith increment validation)

Juvenile weakfish were captured in Delaware Bay in August, 1987, maintained in the laboratory in a recirculating seawater system under a photoperiod of 14 h light/10 h dark at 22°C (0.20 SE), 20‰, and fed ad libitum on squid. Fifty-eight fish were injected with a 200-mg oxytetracycline hydrochloride/0.1 mL saline solution and held in recirculating seawater for 26 days. Throughout the 26-d period, between 1 and 5 fish were removed and measured (SL), and their otoliths were removed for analysis. Fish sizes ranged from 68 to 150 mm SL.

Experiment 2 (effect of ration level on increment width and specific growth rate)

Weakfish were reared from eggs fertilized in the laboratory and raised to the juvenile stage in recirculating seawater (photoperiod=14L/10d at 22°C, 20‰). Individual fish were held in 20-L circular containers and fed ad libitum for two days to determine maximum ration (pretreatment period). Each day, fish were fed a known weight of live mysid shrimp (*Neomysis americana*) in excess of what they could consume. Fish were allowed to feed for 24 hours whereupon uneaten mysids were collected and weighed. Maximum ration was determined to be approximately 20% body weight/d.

Experimental treatment rations were randomly assigned on the third day of the experiment. Fish were weighed to the nearest 0.1 g and randomly assigned one of six daily rations: 100% maximum ration (MR, n=5), 90% MR (n=4), 80% MR (n=4), 60% MR (n=4), 40% MR (n=4), 20% MR (n=4, Table 1). Feeding levels were also calculated as percentages of body weight for individual fishes. For 14 days, fish were fed daily at these assigned levels; that is to say, they were allowed to feed for 24 h whereupon uneaten mysids were removed and collectively weighed. Fish were reweighed on day 7, and final weights and lengths were measured on day 14. The absolute weight of the daily feeding level offered (as a percentage body weight) was adjusted on day 7 to account for growth and maintain ration levels as a function of fish weight. Specific growth rate (SGR) was calculated for each fish as

$$SGR = [(\ln W_{14} - \ln W_0)/14] \times 100,$$

where W_{14} = the wet weight (g) on day 14;
W_0 = the initial wet weight (g); and
14 = the duration of the treatment period in days.

Mean specific growth rates were calculated for each treatment. Fulton's condition factor (K) at the end of the experiment was calculated for each fish as

$$K = W/L^3 \times 10{,}000,$$

where W = the wet weight (g); and
L = the standard length (mm).

Daily ration (percentage body weight/d) was calculated for each fish for each day on the basis of the

Table 1

Summary of ration levels. Actual treatment feeding levels and daily ration calculated based on calculated daily fish weights.

Feeding levels (% of maximum ration)		Daily ration (% body weight/day)		
Estimated feeding level	Actual feeding level	Mean	Week 1	Week 2
100	100	23.8	26.4	21.3
90	66	15.6	16.1	15.0
80	58	13.9	14.6	13.3
60	46	11.0	11.1	10.9
40	32	7.6	7.7	7.5
20	17	4.1	4.1	4.1

weight of mysids consumed (weight of mysids offered minus weight of mysids not eaten) and estimated fish weights (assuming exponential growth between weighing). Mean daily ration was calculated for each feeding level treatment (Table 1). Henceforth, feeding will refer to daily ration, whereas treatment levels will continue to be referred to as percentage of maximum ration (MR).

Because measurements of feeding depended upon the reliability with which uneaten mysids were collected, retrieval efficiency was determined. Live mysids, in amounts comparable to the feeding levels described above, were weighed to the nearest mg and placed in all ten containers with recirculating seawater. After 24 hours, the mysids were retrieved and reweighed. Mean weight of retrieved mysids was 89% (0.017 SE) of initial weight. Therefore, differences between the weight of mysids provided and the weight retrieved was considered to be a useful estimate of feeding.

Otolith preparation and analysis

Otoliths were ground by hand following modified procedures of Neilson and Geen (1981) and Volk et al. (1984). Both sagittal otoliths were embedded in EPON resin. Otoliths were attached to a glass slide with thermoplastic and ground to half their thickness across a transverse plane by using a series of 400–600 grit carborundum paper. Otoliths were polished with 0.3 µm alumina oxide paste, reattached to a glass slide with the polished side down, then ground and polished to produce a thin section through the nucleus. All counts and measures were made from the origin along the dorsal edge of the neural groove to the otolith margin. All other transects lacked precision.

Tetracycline-marked otoliths were examined with UV light at 400× magnification. Increment counts were made from the fluorescent mark to the edge of the otolith. Each otolith was counted twice, without knowledge of the previous measurement, and confirmed by an independent counter. Otoliths from experiment 2 were examined under 400× magnification with transmitted light. Mean IW was calculated from three "blind" measurements. We made all counts and measurements with an Olympus Cue 2 Image Analysis System. One pair of otoliths from the 66% ration treatment was not readable and was subsequently discarded.

Statistical analyses

Experiment 1—daily otolith increment validation To validate the daily nature of otolith increment, the regression slope of increment count on day was tested to determine if it differed from one (Students' t-test). Outliers were detected by calculating the leverage coefficients and by computing the standard residuals from the regression equation line. Only 2% of the otoliths were reexamined because the leverage coefficient was greater than $4/n$ and the standard residual was greater than the t-value for a sample size of n (Sokal and Rohlf, 1981).

Experiment 2—effect of ration level on increment width and specific growth rate Mean IW among ration treatments for increments formed during the pretreatment period was compared with ANOVA (α =0.05), followed by Tukey's multiple comparison tests (Zar, 1984). Mean IW among ration treatments for weeks 1 and 2 and between weeks within ration treatments was analyzed with two-way ANOVA (α =0.05). Mean specific growth rate for each treatment was regressed against mean increment width following confirmation of normality (Kolmogorov-Smirnov test) and homogeneity of variances (Cochran's C test) with α=0.05 for all treatment levels. Increment width was compared with SGR, daily ration, and Fulton's K at the end of the experiment for each fish (Pearson product-moment). Regression analysis was used to determine the relation between IW and SGR (log[G+1]) for each fish. Regression lines were fitted by using a second-order regression against daily ration (Zar, 1984).

Results

Experiment 1—daily otolith increment validation

The slope of the regression increments on days after injection was not significantly different from one (y=0.975x + 0.825, P>0.05), thus supporting the daily periodicity of otolith increment formation in this species (Fig. 1).

Experiment 2—effect of ration level on increment width and specific growth rate

No differences were found in mean IW among treatments during the 2-d pretreatment period. Initially, IW narrowed in the lower feeding levels: 17%, 32%, and 46% maximum MR (Fig. 2). Mean daily IW ranged from a low of 2.3 µm (17% MR or 4.1% body weight/d) during week 2 to a high of 4.5 µm (66% MR or 15.6% body weight/d) during week 1 (Table 2).

Mean IW was significantly lower for the 17%, 32%, and 46% MR treatments during the entire 14-d period as compared with the higher ration treatment

(Table 2; Fig. 3). Increments in the higher ration treatments remained relatively wide throughout the experimental period. Narrowing of increment width in the 17% and 32% MR treatments ensued immediately and continued to decrease after the first week of the experiment (Fig. 2). For all treatments, mean IW was lower during week 2 than week 1; significant differences occurred between weeks in the 17%, 32%, and 66% MR treatments (Table 2). By week 2, there were significant among-treatment differences in mean IW among the 46% MR and the 32% and 17% MR treatments. Mean IW among the higher feeding levels did not differ throughout the entire experiment except for the 66% MR level. During several days of the first week, this group had a significantly higher mean IW compared with other treatments (Table 2). Daily variability in IW was high in all treatments (Fig. 2).

There was a positive correlation ($IW = 2.58 + 1.49(\log\{G+1\})$, r=0.86, P<0.05) between mean daily IW and mean specific growth rate for each treatment (Fig. 4). Although some fish lost weight at the lowest ration level, daily increments continued to be produced. There was a positive correlation between mean IW and mean daily feeding rate and between mean specific growth rate and K at day 14 (Table 3; Figs. 3 and 4). This relation (P<0.05) developed during week 1 of the experiment and strengthened during week 2 (Table 3).

Table 2

Results of two-way analysis of variance and multiple-range tests from comparisons of mean weekly increment width from different ration treatments and one-way analysis of variance between weeks. MR is the maximum ration. * = P<0.05, ns = not significant; letters indicate the results of Tukey's HSD comparison among treatments. SE is the standard error associated with treatment means.

Treatment feeding levels (% MR)	Week 1	SE	Week 2	SE	Treatment means
17	3.042	0.0243	2.310	0.0378*	2.68^{a}
32	3.021	0.0465	2.616	0.1203*	2.82^{ab}
46	3.121	0.1402	3.027	0.1495ns	3.07^{b}
58	3.823	0.7427	3.817	0.5864ns	3.82^{c}
66	4.514	0.2835	3.766	0.8348*	4.14^{c}
100	3.864	1.2942	3.755	0.6493ns	3.81^{c}
Overall	3.538		3.215*		3.38

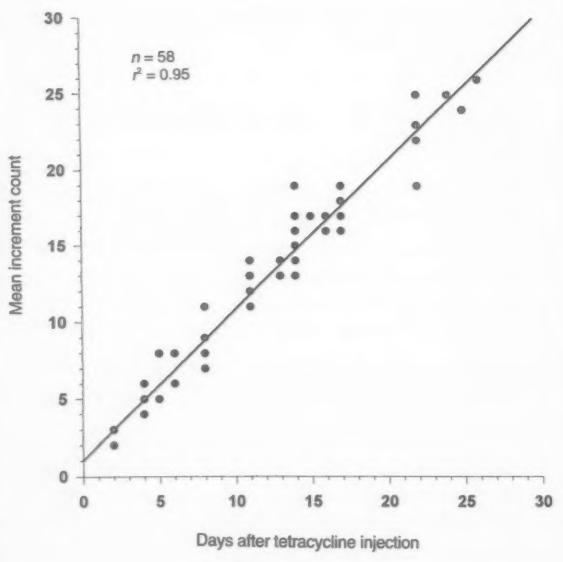

Figure 1

Relation between otolith increments distal to the fluorescent mark and days after tetracycline injection in juvenile weakfish, *Cynoscion regalis*. Symbols may represent more than one observation.

Discussion

Injection of oxytetracycline hydrochloride solution produced clear fluorescent bands in the otoliths of juvenile weakfish, and increment deposition occurred daily. Several other juvenile sciaenids have shown daily increments: spot (*Leiostomus xanthurus*), red drum (*Sciaenops ocellatus*), spotted seatrout (*Cynoscion nebulosus*), and silver perch (*Bairdiella chrysoura*) (Gjosaeter et al., 1984; Hettler, 1984; Peters and McMichael, 1987; McMichael and Peters, 1989; Hales and Hurley, 1991). The present study provides validation for ageing juvenile weakfish, thus enabling estimates of growth and providing, in combination with abundance data, a means of estimating accurate age specific mortality rates during this life history stage.

The rapid response of IW to changes in ration and the strong relation with SGR suggest that IW's may be used to infer growth history. Furthermore, significant differences in IW between high (58–100% MR) and low (17–46% MR) feeding levels suggests that IW may be used to approximate feeding history. Because of approximately a one-week lag time prior to stabilization of IW among treatments, the full magnitude of the change in IW cannot be assessed by examining just a few increments. At

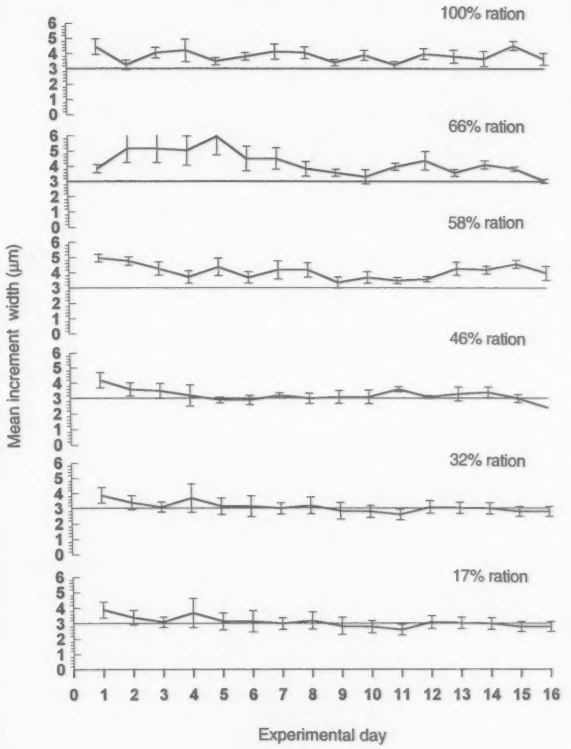

Figure 2

Relation between mean daily otolith increment width and experimental day for all feeding levels. Feeding levels are expressed as a percentage of maximum daily ration. Error bars represent ± SE.

least one week is required before IW responses to feeding and growth are statistically detectable although the physiological processes that result in IW differences begin acting sooner (Molony and Choat, 1990). Therefore, mean IW taken over several consecutive days would be most useful for making inferences regarding recent feeding and growth history for small sample sizes.

The magnitude of variability in IW observed in this study, particularly for the higher rations, has been documented for other species (Volk et al., 1984; Neilson and Geen, 1985; Maillet and Checkley, 1991). The reduced variability under the stress of lower ration may be related to reduced growth and utilization of food and stored reserves for maintenance (Molony and Choat, 1990). Continuation of otolith increment formation during periods of negative fish growth suggests that otolith growth is conservative and otolith resorption is not likely (Campana and Neilson, 1985; Secor et al., 1989).

Table 3

Results of Pearson product-moment correlation analysis (r) between daily increment width, daily feeding rate, and specific growth rate.

	Increment width		
	r	n	$P<0.05$
Daily feeding rate			
Week 1	0.5100	168	0.000
Week 2	0.6552	168	0.000
Specific growth rate			
Week 1	0.3961	168	0.000
Week 2	0.6232	168	0.000
Condition factor	0.3576	336	0.000

Otolith increments of spot, like those of weakfish, were found to have an immediate response to changes in ration (Govoni et al., 1985), although deposition

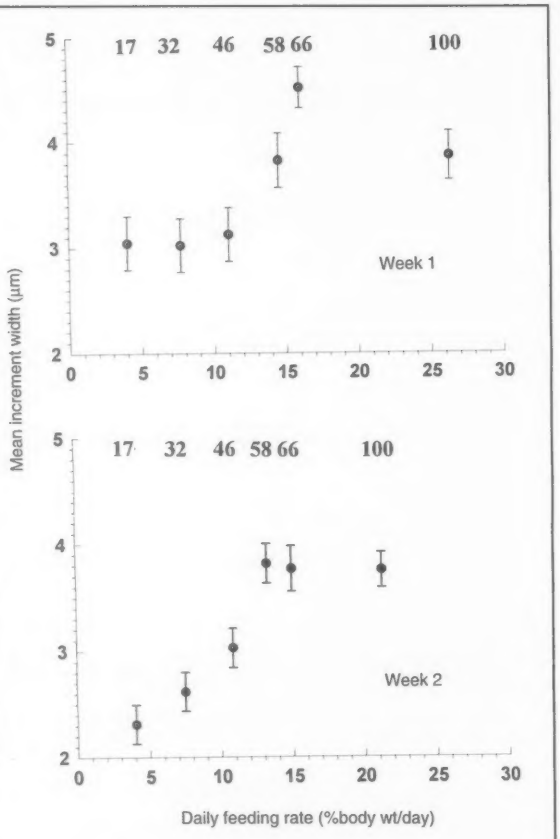

Figure 3

Relation between mean daily otolith increment width and daily feeding rate in juvenile weakfish, *Cynoscion regalis*, during week 1 and week 2. Error bars represent 95% Tukey's multiple comparison intervals. Values along the top of each panel are treatment feeding levels expressed as a percentage of maximum daily ration.

was found to be less than daily under low ration conditions (Siegfried and Weinstein, 1989). However, for the bloater (*Coregonus hoyi*) there was a loss of contrast between the hyaline and opaque bands and no effect on relative increment width (Rice et al., 1985). Maillet and Checkley (1990) found that starved Atlantic menhaden (*Brevoortia tyrannus*) larvae produced narrower increments with IW, increasing during a 3–6 day recovery period. In contrast to these examples of rapid otolith response to ration, changes in IW in juvenile chum salmon (*Oncorhynchus tshawytscha*) and the tropical glass fish (*Ambassis vachelli*) do not become discernible for three weeks and two weeks, respectively (Neilson and Geen, 1985; Molony and Choat, 1990). Recent experimental data suggest that the IW-growth relation may be more complex than originally thought (Reznick et al., 1989; Secor et al., 1989; Francis et al., 1993; Jenkins et al., 1993). The result of these studies suggests that the IW response to feeding and growth is variable, may be of limited use in some species, and needs to be evaluated on a species by species basis.

For species in which the relation of IW to growth has been established, otolith increment analysis can provide a means by which an investigator may relate recent environmental conditions to recent growth history during the important early life stages. Thus, a more complete understanding of the role of the environmental conditions relating to feeding, growth, and ultimately survival may be obtained.

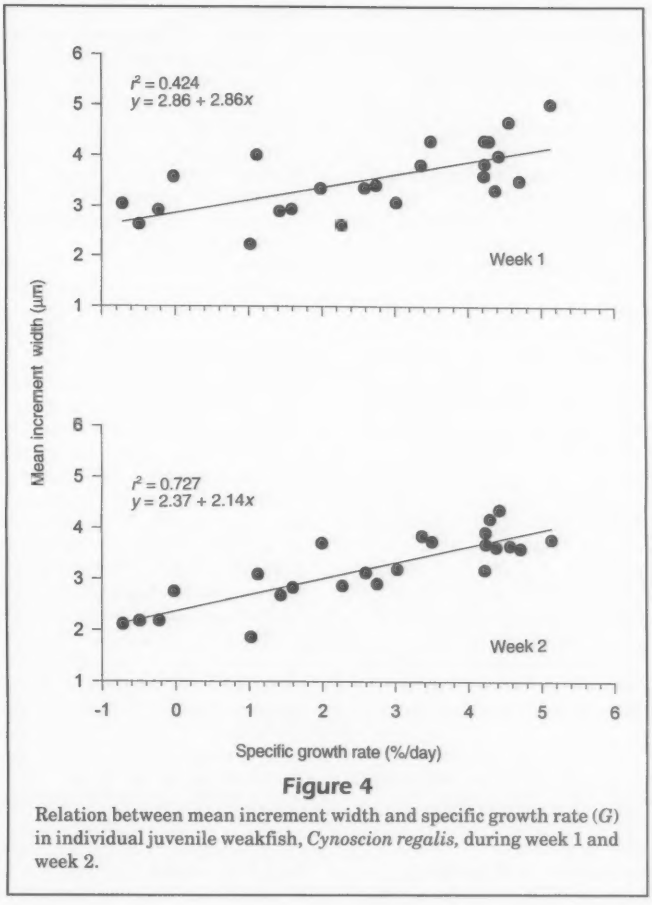

Figure 4

Relation between mean increment width and specific growth rate (G) in individual juvenile weakfish, *Cynoscion regalis*, during week 1 and week 2.

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Daily age and growth of larval and early juvenile Spanish mackerel, *Scomberomorus maculatus*, from the South Atlantic Bight*

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Abstract.—Age and growth of larval and juvenile Spanish mackerel, *Scomberomorus maculatus*, were determined by examining increments of daily growth on the otoliths (lapilli) of specimens collected along the southeastern Atlantic coast, 1983–89. Marginal increment analysis was performed on 152 fish (7.4–97.0 mm SL) to validate the deposition of daily rings. A mean standardized marginal increment (SMI) was calculated by comparing the width of the marginal increment to the adjacent increment on the lapilli of fish captured over a diel cycle. The distribution of mean SMI was unimodal. A nonlinear equation was used to model growth ($\ln SL = 6.2 - 55.1/Age$). Based on this growth equation, predicted absolute growth rates for the first 23 days of life were approximately 1.9 mm/day, followed by a surge of rapid growth approaching 5.0 mm/day over the next 17 days. Absolute growth rates subsequent to 40 days of age were 2.1 mm/day.

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Fishery Bulletin: 530–539 (1997).

The Spanish mackerel, *Scomberomorus maculatus* (Mitchill), is an inhabitant of the Gulf of Mexico and the Atlantic coast of the United States. During winter months, Spanish mackerel are concentrated in waters off southern Florida. In late spring and summer, however, they are widely distributed along the Atlantic coast to the Gulf of Maine (Klima, 1959; MacEachran et al., 1980; Finucane and Collins, 1986).

Most life history studies on Spanish mackerel have focused on adults from southern Florida and the Gulf of Mexico (Klima, 1959; Powell, 1975; Finucane and Collins, 1986; Fable et al., 1987; Schmidt et al., 1993). Except for work by DeVries et al. (1990) on growth rates of larval and early juvenile Spanish and king mackerel (2.8–22.0 mm SL), very little has been done on the early life history of *S. maculatus*, particularly in the South Atlantic Bight (SAB).

Daily growth increments on otoliths of juvenile scombrids (skipjack, *Euthynnus pelamis*, and yellowfin tuna, *Thunnus albacares*, bluefin tuna, *T. thynnus*, black skipjack, *Euthynnus lineatus*, Atlantic mackerel, *Scomber scombrus*, and southern bluefin tuna, *Thunnus maccoyii*) have been tentatively validated (Uchiyama and Struhsaker, 1981; Radtke, 1983; Wild and Foreman, 1980; Brothers et al., 1983; D'Amours et al., 1990; Jenkins and Davis, 1990; Wexler, 1993). However, no published study has been directed at the validation of daily growth increments on the otoliths of Spanish mackerel.

The validation of the consistent periodic deposition of growth rings generally requires that fishes be held in captivity under conditions that approximate the natural environment. However, Spanish mackerel larvae and juveniles are difficult to rear in the laboratory. Another method that has moderate reliability involves demonstrating that initiation of increment formation is synchronous throughout the population (Tanaka et al., 1981; Geffen, 1987; Jenkins and Davis, 1990). If fishes deposit increments in response to external environmental cues of diel periodicity, or an endogenous daily rhythm, then individuals experiencing the same environmental conditions (light, temperature, feeding activity) would be expected to initiate increment deposi-

* Contribution 377 from the South Carolina Department of Natural Resources, Charleston, South Carolina 29422 and contribution 135 from the University of Charleston's Grice Marine Laboratory, Charleston, South Carolina 29412.

tion at approximately the same time of day. A review of this approach is presented in Tanaka et al., 1981; Brothers and MacFarland, 1981; and Geffen, 1987.

The age and growth data used in this paper came from two separate studies, one dealing primarily with larvae and small juveniles less than 100 mm SL, the other with larger young-of-the-year (YOY) juveniles. The primary objectives of this paper are to combine these studies to present a more comprehensive analysis of age and growth of larval and juvenile (7–353 mm SL) Spanish mackerel and to validate the daily deposition of increments on their otoliths.

Methods

Collection and treatment of specimens

Past studies attempting to describe the age, growth, and distribution of Spanish mackerel have resulted in the collection of a relatively small number of specimens over a limited size range (MacEachran et al., 1980; Collins and Stender, 1987; DeVries et al., 1990). Because of the apparent difficulty in capturing Spanish mackerel larvae and juveniles, we attempted to increase our sample size by pooling ancillary collections of Spanish mackerel from unrelated studies when they became available. This allowed us to use a wide size range of specimens collected over an entire diel cycle.

Most of the Spanish mackerel larvae and juveniles (7.4–353.8 mm SL) were collected with a 1 m × 2 m neuston net (2.0 mm mesh) from Breach Inlet bridge, near Charleston, SC, during the entire nighttime flood tide (Fig. 1). The sampling effort was designed by the South Carolina Department of Natural Resources (SCDNR) to capture larval gag that enter the estuaries during the spring of each year. Spanish mackerel were obtained from samples that were taken during the month of June from 1986 through

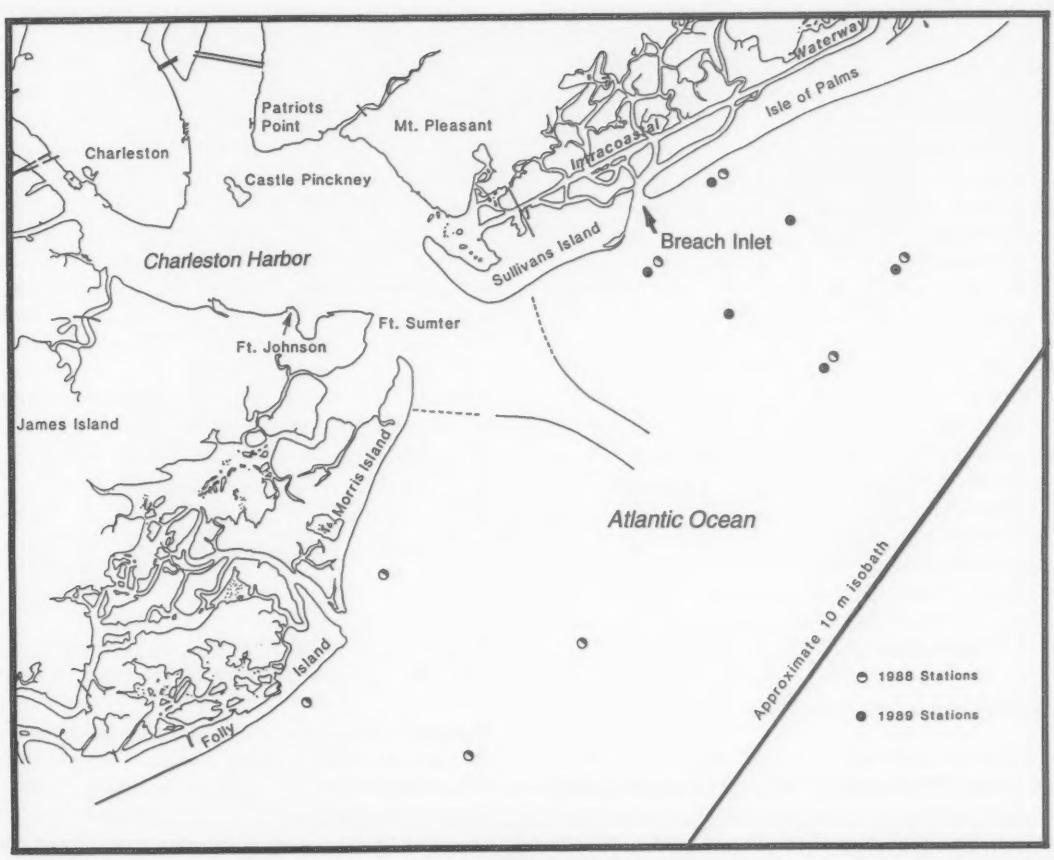

Figure 1

Locations of nearshore ichthyoplankton stations sampled during 1988 and 1989 and location of Breach Inlet, off Charleston, SC, where sampling was done for this study.

1988. In addition, 25 larval and juvenile Spanish mackerel were collected in ichthyoplankton samples from coastal waters off Charleston, SC, during May–October 1988 and 1989 (Fig. 1). During 1988, samples were obtained with a 0.5 m × 1 m (0.505-mm mesh) side-towing neuston net. The 1989 samples were taken with a 1 m × 2 m (0.947 mm mesh) neuston net. In addition, larger juvenile Spanish mackerel (> 60 mm SL) were obtained during 1983–89 along the coast of North Carolina, South Carolina, and Georgia from SCDNR research cruises aboard the RV *Oregon* and RV *Lady Lisa* with trawls, gill nets, seines, and from commercial shrimp trawling bycatch (Collins et al., 1988; Beatty et al.[1]).

Larvae and juveniles (<100 mm SL) were preserved in 95% ethanol and measured (standard length [SL], fork length [FL], and total length [TL]) to the nearest 0.1 mm with dial calipers or ocular micrometer (Wild, M5 dissecting scope). Owing to the poor condition of the caudal fin on many of the smaller fish, standard length was used in the age and growth analysis. A factor of 3% was added to the length of each fish to account for shrinkage in ethanol (Schmidt, unpubl. data, 1988). All fish were identified following Wollam (1970) and Richardson and MacEachran (1981). Sagittae and lapilli were excised from larvae and small juveniles by immersing the head region in 5% sodium hypochlorite solution for no more than 30 minutes (Brothers, 1987). Otoliths were separated from undissolved tissue and bone under a dissecting microscope with transmitted crossed polarized light. Otoliths from larger juveniles were removed by dissecting out the entire otic capsule and by separating the otoliths from their respective ampullae. Excess tissue was dissolved in sodium hypochlorite solution. Otoliths were then rinsed in water, mounted whole (concave side down, unpolished) in immersion oil on a microscope slide and examined on a video-enhanced (Hitachi, MOS) compound microscope (Nikon, Labophot). Lapilli were used to estimate age in Spanish mackerel larvae and juveniles because increments were more discernible in the lapilli than in the sagittae. Young-of-year juveniles (>100 mm SL) were treated according to the same procedures used for YOY king mackerel by Collins et al., 1988.

Marginal increment analysis

To confirm the hypothesis of daily increment deposition, a marginal increment analysis was performed. In this analysis, the stage of completion of the marginal increment was compared with the adjacent fully formed increment on the lapilli from fish captured over a daily cycle (Fig. 2). Because Breach Inlet specimens were captured over an entire flood tide, it was impossible to know their precise time of capture. Therefore, the mean stage of completion of the marginal increment of several specimens, captured over 5–6 hour periods that progressed throughout the day and night, was compared. Large collections were subsampled by selecting as many as 35 individuals representing the size range of fish captured in the sample. Additional mackerel taken in SCDNR trawls and nearshore ichthyoplankton samples were also used. The time of capture of these specimens was known to within 30 minutes. A total of 165 larval and juvenile Spanish mackerel (7–97 mm SL) were examined. Attempts to find evidence for the daily nature of otolith rings in larger juveniles by measuring diel variation in marginal increments with SEM were not successful.

Measurements of the marginal increment and the adjacent increment were made along each of three separate axes on each otolith. These axes were chosen because their optical properties allowed acceptable ring resolution. Occasionally, it was not possible to measure all three axes owing to opacity or damage to the otolith. Increments were displayed on a video monitor at 1,000× and measured to the nearest 0.1 mm with dial calipers. Care was taken in observing the opaque and transparent zones because different focal planes may invert their appearance. Consistent counts and marginal increment measurements were obtained at a "high" focal point (the distance [with the highest lens power] to object that will produce a well-defined image). We were unaware of time of capture while performing the measurements. A standardized marginal increment (SMI) for each axis of measurement was calculated as

$$SMI = \frac{W_n}{W_{(n-1)}},$$

where W_n = width of marginal increment; and
$W_{(n-1)}$ = width of complete adjacent increment.

The SMI's for each of the axes were averaged to obtain a mean SMI for each otolith. Two independent mean SMI's were calculated for each otolith from separate measurements. Although there was no significant difference between the two measurements (paired t-test, P=0.153), the second measurement was used in the analysis because we were more experienced at locating and measuring the marginal increment.

[1] Beatty, H. R., J. W. Hall, and E. L. Wenner. 1988. Results of trawling efforts in the coastal habitat of the South Atlantic Bight 1987–1988. South Carolina Division of Natural Resources, P.O. Box 12559, Charleston, SC 29422. SEAMAP Report, 94 p.

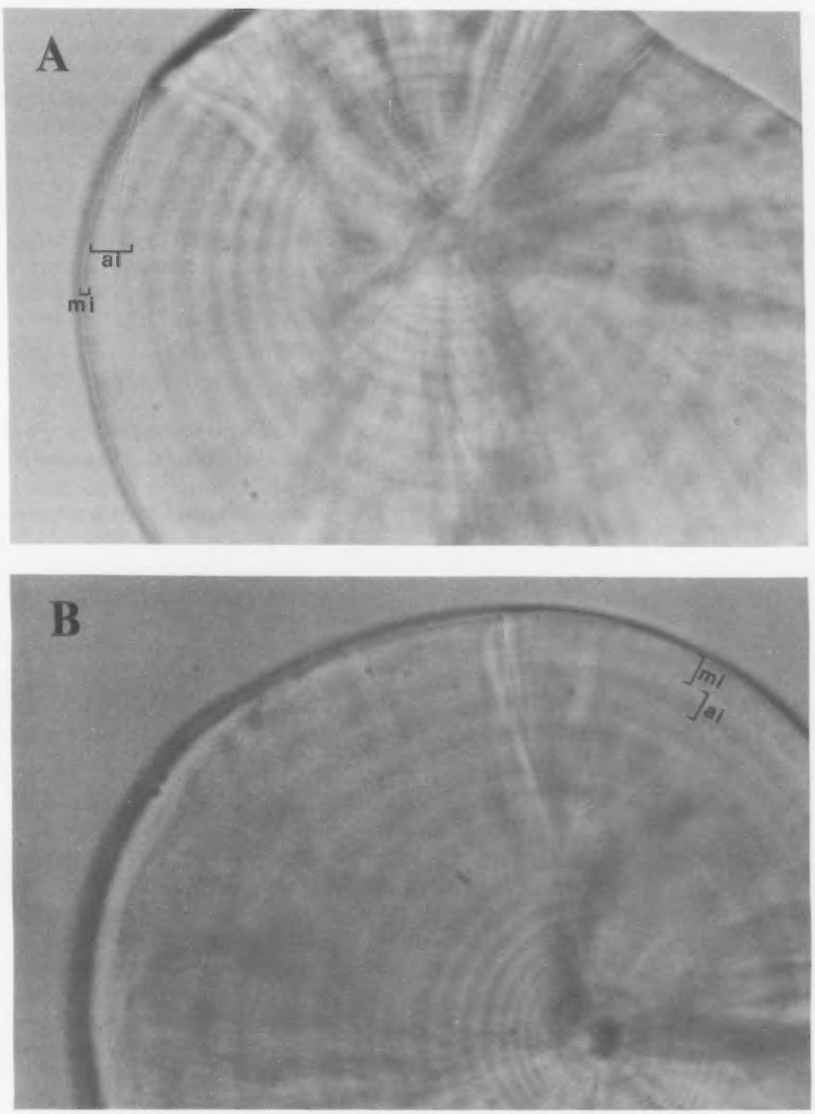

Figure 2

(**A**) Lapillus from a Spanish mackerel juvenile (SMI=0.3) captured at Breach Inlet between 0309 h and 0830 h EDT. (**B**) Lapillus from a Spanish mackerel juvenile (SMI=0.9) captured at Breach Inlet between 1613 h and 2211 h. Incomplete marginal increment (mi), and adjacent fully formed increment (ai) are indicated.

Age and growth analysis

Whole lapilli from 415 larval and juvenile Spanish mackerel were examined. For larvae and juveniles <100 mm SL, otolith radius was measured from the center of the primordium to the margin of the otolith along a consistent axis. Measurements were made with an ocular micrometer at magnifications of 100× or 400× depending upon the size of the otolith. Presumed daily increments on the lapilli were counted on a video monitor under 1,000× magnification. Two independent counts of presumed daily increments

were made; we were unaware of fish length and any prior age determination during counting. Incomplete marginal increments were not counted. Furthermore, counts of right and left otoliths were conducted separately. In situations where the first two counts differed, a third independent count was performed. The assigned age corresponded to the two counts that were in agreement. If agreement could not be reached on two of the three counts, the otolith was considered unreadable and was not used. Otoliths in YOY juveniles (>100 mm SL) were counted according to the procedures used for YOY king mackerel in Collins et al. (1988).

Nonlinear regression analysis was used to describe the relation between age and length. Statistical analyses were performed with SYSTAT software (Wilkinson, 1988) and Table Curve (Jandel Scientific) and were based on a significance level of 0.05.

Results

Several features of increment deposition were observed to be consistent among the otoliths examined. Two diffuse and poorly defined increments (core increments) surrounded the primordium (Fig. 3). Mean core width was 11.4 mm and there was little variation with fish length (SD=0.54 mm, n=40, length range=9.0–300.1 mm). Although these increments were counted as daily, the nature of their deposition was clearly different from that of subsequent rings. This finding indicated that they were formed during a separate developmental stage. The absence of fish younger than 9 days precluded precise determination of the time period represented by these two increments. Subsequent increments were clearly defined on most lapilli and were easily discernible in whole otoliths examined under a light microscope without any special preparation (grinding or polishing). Subdaily increments occurred, particularly in older juveniles, and were discernible from the daily increments (Fig. 4).

Marginal increment analysis

Of 165 fish examined for marginal increments, 13 were not used in the final analysis owing to damage to the otoliths or to uncertainties in distinguishing the marginal or adjacent increments (or both). No significant difference in SMI was found between left and right lapilli (paired t-test, P=0.191). Examination of fish captured during the 1613–2330 h time period revealed an obvious split in the stage of marginal increment completion (Table 1). A unimodal distribution of mean SMI, for fish captured over a 24-h period, was obtained if the mean SMI of those otoliths whose margin was bordered by a translucent zone

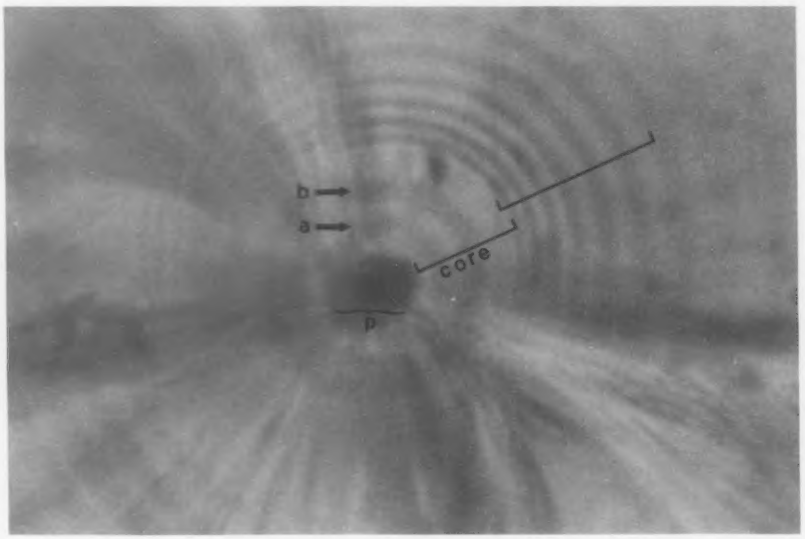

Figure 3

Photomicrograph of the core region with two diffuse, atypical increments (a and b), surrounding the primordium (p), and first six presumed daily increments (brackets) on a Spanish mackerel lapillus.

(late stage of increment formation) was plotted separately from the mean SMI of otoliths whose margin was bordered by an opaque zone (early stage of increment formation) (Fig. 5). The observed separation in the stage of increment formation would be expected if initiation of increment formation occurred between 1613 h and 2330 h.

Age and growth

Otolith radii measurements revealed no significant difference between right and left lapilli (paired sample t-test, P=0.127). The relation between SL versus lapillus radius was described by the following regression equation:

$$SL = -4.78 + 0.68(Radius) \quad [r^2=0.99, n=364].$$

No significant difference was found between increment counts for left and right lapilli (paired t-test, P=0.190). Therefore, if left and right counts differed, the otolith whose increments were more clearly defined, or which was in better condition, was used to assign an age to the fish. Growth in young Spanish mackerel was quite variable within age classes, particularly in juveniles older than 23 days (Fig. 6). Nonlinear regression analysis provided the following growth equation:

$$\ln SL = 6.2 - 55.1/Age.$$

Table 1

Mean standardized marginal increment (MSMI), range, standard deviation for left (L) and right (R) lapilli, and size range for *S. maculatus* captured from 1613 to 2211 h and 1755 to 2330 h. Lapilli with opaque margins are considered separately from lapilli with translucent margins. (n refers to the number of specimens, no. refers to the number of right or left lapilli.) Hours are those of eastern daylight time.

	Lapilli from fish collected 1613–2211 h				Lapilli from fish collected 1755–2300 h			
	Opaque		Translucent		Opaque		Translucent	
	R	L	R	L	R	L	R	L
n	34				10			
No.	18	18	16	16	6	6	2	4
MSMI	0.19	0.22	0.88	0.86	0.25	0.23	0.75	0.75
SD	0.05	0.07	0.08	0.15	0.06	0.05	0.07	0.13
Range	0.3	0.2	0.3	0.5	0.1	0.1	0.1	0.3
Size range (SL)(mm)	17.1–97.0				22.6–79.0			

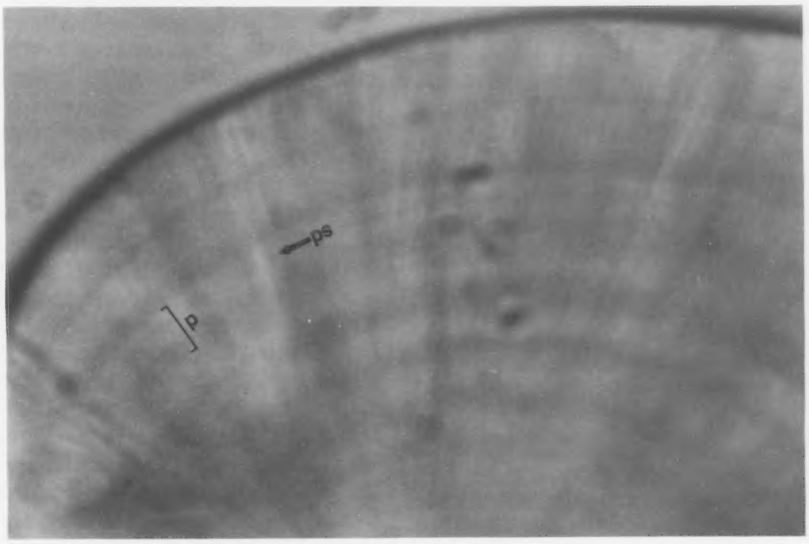

Figure 4

Lapillus of an 18-day-old juvenile Spanish mackerel (18.7 mm SL). Note occurrence of subdaily rings as the fish ages. A daily growth increment (d) and a presumed subdaily increment (sd) are indicated.

Based on this growth equation, predicted absolute growth rate (predicted SL/age in days) was 2.4 mm/day for the first 150 days of life. Early growth was characterized by relatively slow growth for the first 23 days of life (1.9 mm/day) followed by a surge of rapid growth from 23 to 40 days, during which growth rates approached 5.0 mm/day. Predicted absolute growth of older juveniles (40–150 days) was 2.0 mm/day.

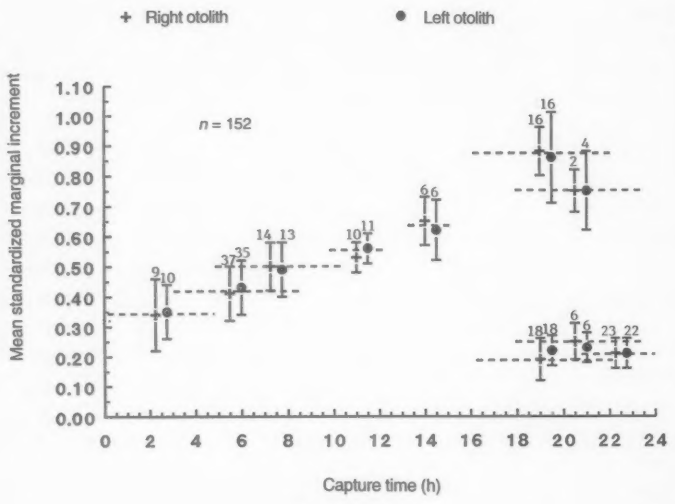

Figure 5

Mean standardized marginal increment and standard deviation for left and right lapilli of Spanish mackerel larvae and juveniles collected throughout the day and night. Mean SMI for opaque bordered marginal increments are plotted separately from translucent bordered marginal increments in samples taken from 1613–2330 h. Capture time ranges (dashed lines) and sample sizes are indicated.

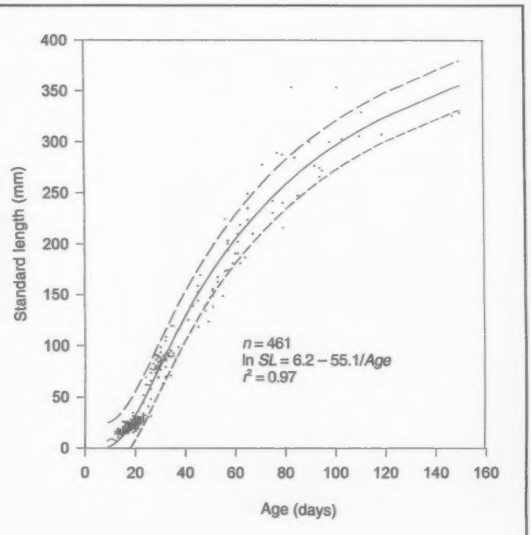

Figure 6

Relation between standard length (SL) and age in days and 95% confidence limits (dashed lines) determined from otolith increment counts for Spanish mackerel.

Discussion

Validation of daily formation of the microstructural increment is a necessary prerequisite to using otoliths for ageing larval and juvenile fishes. Determination of the stage of completion of the most recently formed increment over a daily light cycle does not directly validate daily increment formation but lends strong support to the hypothesis that increments are deposited daily.

Several studies have shown that increment deposition is most likely controlled by an endogenous rhythm that can be modified by physical or behavioral parameters (or both), such as light and dark periodicity, temperature regimes, feeding frequency, food availability, activity patterns (such as daily vertical migrations), or a combination of these and other factors (Jones, 1986; Campana and Neilson, 1985, for review). There is presently no information available on the effects of changes in environmental factors on the periodicity or pattern of increment formation in larval or juvenile scombrids. However, work done with other teleosts (Taubert and Coble, 1977; Tanaka et al., 1981; Campana, 1984; Neilson and Geen, 1985; Jenkins and Davis, 1990) suggests that an internal diel clock alone is not responsible for daily increment formation but that it is entrained by some external environmental cue that can vary between species of fishes.

Observations on the seasonal occurrence and distribution of larval Spanish mackerel in the northern Gulf of Mexico and the South Atlantic Bight suggest that they are restricted to middle and inner continental shelf waters (Dwinell and Futch, 1973; MacEachran et al., 1980; Collins and Stender, 1987). Since daily fluctuations in salinity and turbidity are minimal in shelf waters outside estuarine influence, they are not likely to modify cyclic daily deposition of increments in larval Spanish mackerel. It seems more likely that feeding periodicity or diel vertical

migrations, which are often strongly associated with light cycles, serve to increase daily increment definition (Campana and Neilson, 1985).

Other species of scombrid larvae and early juveniles (*E. pelamis*, *T. albacares*, *E. alletteratus*, *Auxis thazard*, and *Scomber scombrus*) are known to undergo vertical diel migration feeding primarily at the surface at night (Matsumoto, 1958; Grave, 1981). Collins and Stender (1987) found statistical evidence for vertical migration to the surface at night in both *S. maculatus* and *S. cavalla*. Spanish mackerel and other species of *Scomberomorus* are known to feed on ichthyoplankton during the larval stage and are almost completely piscivorous as juveniles (Naughton and Saloman, 1981; Jenkins et al., 1984). The large eyes of scombrids, even during the larval stage, suggest that they are visual predators. Therefore, light cycles probably have a strong influence on prey detection. Moreover, feeding opportunities related to diurnal vertical migrations of prey organisms, along with fluctuations in temperature associated with diel vertical migrations, may further serve to entrain this endogenous rhythm of calcium carbonate deposition.

Age and growth

Marginal increment analysis indicated that otolith increments are deposited daily in larvae and juveniles from 7 to 95 mm SL. However, because we were unsuccessful at capturing preflexion larvae, it was impossible to determine if increment counts truly reflected age from fertilization. Very little information is available on otolith formation in scombrids, although otoliths are among the first calcified structures and are present in scombrid embryonic stages (Matsumoto, 1958; Radtke, 1983; Brothers et al., 1983). In *E. pelamis* larvae reared from hatching (Radtke, 1983), the core region of the otolith (the primordium and two diffuse increments), along with the pattern of subsequent increment formation, is very similar in appearance to otoliths of *S. maculatus* (Fig. 3). Radtke (1983) observed that the two core increments were present at hatching.

The two core increments in *S. maculatus*, because of their atypical pattern of deposition, are likely to have been formed during the egg stage or prior to yolk-sac absorption. However, it is not known whether these increments are deposited daily. Because hatching and yolk-sac absorption of Spanish mackerel larvae usually occurs five days after fertilization at temperatures experienced during the spawning season in South Carolina waters (Berrien and Finan, 1977; Fritzche, 1978), errors in age estimation are likely to be consistent among most fish, and growth rate calculations would remain unaffected.

Considerable variation was observed in the growth rates of individual fish, particularly among juveniles older than 23 days. The use of specimens collected over a wide spatial and temporal range was probably responsible for much of this variation. However, the overall predicted mean absolute growth rate of 2.4 mm/day is within the range of growth rates observed in other scombrids during the first few months of life (1 mm/day–6 mm/day) (see Brothers et al., 1983, for review). The regression lines estimating the relationship between age and length appeared to be a good approximation (r^2=0.97, P<0.0001) of growth in young Spanish mackerel.

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Abstract.–Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope measurements were used to differentiate groups of king mackerel, *Scomberomorus cavalla*, in the northwestern Gulf of Mexico and off the southeastern coast of Florida, as well as off the coast of Mexico. Northwestern (+13.1‰) and southeastern (Mexico=+10.8‰ and Florida=+10.8‰) groups, as well as the Atlantic group, had significantly different stable nitrogen isotope ratios. These were attributed to isotopic variations at the base of the food chain. Variability in $\delta^{13}C$ measurements was too large and did not corroborate the $\delta^{15}N$ results. The grouping suggested by the $\delta^{15}N$ data can be explained by the influence of the Mississippi River and the Gulf of Mexico Loop Current.

Use of stable isotopes to assess groups of king mackerel, *Scomberomorus cavalla*, in the Gulf of Mexico and southeastern Florida

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King mackerel, *Scomberomorus cavalla*, is one of the most sought after migratory pelagic resources in waters of the contiguous United States (Dwinell and Futch, 1973; Manooch et al., 1978; Manooch, 1979; Finucane et al., 1986). This species is strongly exploited by both sport and commercial fisheries, and fishing pressure may exceed maximum sustainable yields of the Gulf resource (Gulf of Mexico and South Atlantic Fishery Management Councils[1]). In the United States, commercial catches of king mackerel were 2,013 metric tons (t) in 1994 (U.S. Dep. Commer., 1995). Recreational catches in the United States are thought to be larger than commercial landings (Deuel and Clark, 1968; Deuel, 1973; Manooch, 1979; U.S. Dep. Commer., 1985–1987). Recreational catches are reported as individuals rather than as weight; however, an estimated total weight of recreational catches for 1991 was 2,713 t (U.S. Dep. Commer., 1992).

Current management plans are based on a two-stock model, an Atlantic stock and a Gulf of Mexico stock (Gulf of Mexico and South Atlantic Fishery Management Councils[1]). However, general consensus among scientists is that two stocks of king mackerel exist within the Gulf of Mexico. Evidence for two stocks within the Gulf of Mexico includes fisherman observations of migration (Baughman, 1941), electrophoretic studies (Grimes et al., 1987; Johnson et al., 1994; May[2]), catch-per-unit-of-effort data from charter boats (Trent et al., 1987), and differences in spawning times (Grimes et al., 1990).

Stable isotopes (C, N) have been used to study trophic levels and feeding strategies of organisms (see Macko et al., 1984; Peterson and Fry, 1987; Koch et al., 1995). According to DeNiro and Epstein (1978), the carbon isotopic composition of a food source is not substantially altered during assimilation. DeNiro and Epstein (1981) found that animals also reflect the nitrogen isotopic composition of their diet; however, there is a trophic-level enrichment. Regardless of habitat, form of nitrogen excreted, and growth rate, an isotopic enrichment of +3.4

[1] Gulf of Mexico and South Atlantic Fishery Management Councils. 1992. Amendment 6 to the fishery management plan for coastal migratory pelagics in the Gulf of Mexico and South Atlantic includes environmental assessment regulatory impact review and initial regulatory flexibility analysis. Gulf Mex. S. Atl. Fish. Manage. Counc., Tampa, FL, var. pagin.

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±1.1‰ occurs for nitrogen isotopes (Minagawa and Wada, 1984). Macko et al. (1984) also demonstrated that stepwise enrichment, which occurs from trophic level to trophic level, does not vary among locations. Finally, Minagawa and Wada (1984) suggested that individuals do not fractionate nitrogen isotopes differently at various ages.

Estep and Vigg (1985) observed that the carbon isotope discrimination between scale and muscle was consistent for a particular fish species, and stated that isotopic measurements in muscle and scales could be used to determine diet of fish. Studies that encompass time scales of months to years, however, may be compromised by fast turnover of muscle. Collagen, in contrast to muscle, has a slow turnover rate of carbon (Libby et al., 1964). Collagen amino acid composition varies only slightly among species (see Schoeninger and DeNiro, 1984); therefore, differences in collagen isotope ratios reflect isotopic changes in diet and not variations in chemical composition (Schoeninger and DeNiro, 1984). Additionally, we speculate that turnover of collagen may be slower in poikilotherms, such as fish, compared with homeotherms, such as mammals, owing to the lower metabolic rate of poikilotherms. The chemical uniformity and slow turnover time of collagen make it a suitable matrix for recording the dietary history of organisms that grow fin spines (fin spines consist of collagen fibers in a bony matrix) and live over periods of years.

In the Gulf of Mexico, Fry (1983) compared stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of several decapod crustaceans and two species of inshore fishes. The study revealed that groups feeding primarily in the eastern Gulf of Mexico have different $\delta^{15}N$ and $\delta^{13}C$ values from those feeding in the western Gulf of Mexico (Table 1). Macko et al. (1984) also reported geographical variations in isotopic ratios of sedimentary organic matter. From these studies, we hypothesized that stable carbon and nitrogen isotopes would aid in establishing group structure if 1) king mackerel feed in different regions of the Gulf of Mexico for extended periods of time, and 2) the carbon and nitrogen isotope composition of a food source is incorporated in a consistent manner into tissues of king mackerel. Dorsal fin spines were chosen for isotopic analyses because collagen has a slow turnover rate, and the isotopic ratio of the spine should reflect assimilated food at the time of formation. Therefore, the diet recorded within the spine is chiefly a record of early developmental years, when the majority of growth occurs. A previous study, however, showed a loss of the first annulus in fin spines of older swordfish (Tsimenides and Tserpes, 1989). We could find no such study of fin spine annulus of king mackerel to substantiate this loss in king mackerel. Loss of mass has not been investigated in swordfish research; therefore, we assumed that if any material is lost, it is minimal in comparison with the remaining material, because this phenomenon has been observed only in large individuals. Below, we report on the identification of two isotopically distinct groups of king mackerel in the Gulf of Mexico and compare our findings with previous studies conducted in order to determine location and number of king mackerel groups.

Table 1

Mean stable isotope (C and N) values of sediment, particulate organic matter, zooplankton, shrimp, and mackerel for Florida, Northwestern Gulf of Mexico, and Mexico. Standard error is presented when available. GOM = Gulf of Mexico. nd = no data. Florida king mackerel data comprise collection sites Panama City, FL, and Fort Pierce-Palm Beach, FL. Northwestern king mackerel data comprise collection sites Port Aransas, TX; Galveston, TX; Grand Isle, LA; and Gulf Port, MS. Mexico king mackerel data comprise collection sites Dzilam DeBravo, Celestun, and Veracruz.

	$\delta^{13}C$			$\delta^{15}N$		
Sample type	Florida	Northwestern GOM	Mexico	Florida	Northwestern GOM	Mexico
Sediment[1]	−18.5 ±0.7	−20.6 ±0.6	nd	3.6 ±0.1	6.5 ±0.2	nd
Particulate organic matter[1]	−19.4 ±1.2	−21.0 ±1.4	nd	−0.9 ±1.4	7.5 ±0.8	nd
Zooplankton[1]	−18.4 ±1.1	−19.2 ±0.7	nd	5.9 ±0.7	8.9 ±0.9	nd
Penaeus shrimp[1]	−14.8 ±0.5	−15.6 ±1.1	nd	8.4 ±0.9	12.9 ±1.1	nd
Penaeus shrimp[2]	−14.6	−15.9	nd	8.3	12.6	nd
King mackerel (this study)	−18.9 ±1.1	−18.1 ±0.9	−17.7 ±1.5	10.8 ±1.1	13.1 ±1.3	10.8 ±1.0

[1] Data, excluding the king mackerel data, were compiled from Fry (1983) and Macko et al. (1984).
[2] Data are based on estimated values from Figure 7 in Fry (1983).

Methods and materials

King mackerel were obtained from the Southeast Fisheries Center, National Marine Fisheries Service, Panama City, FL, and laboratory of John R. Gold, Department of Wildlife and Fisheries Sciences, Texas A&M University. These samples were collected within the Gulf of Mexico and Atlantic Ocean (Fig. 1) during November and December 1991; February, May, and July 1992; and March, May, and June 1993 (Table 2). Two to twenty fish were analyzed per site (average of seven per locality). Locality, date collected, fork length (standard measure of size), weight and sex of the fish specimens were recorded for most samples. According to the length-at-age study of DeVries and Grimes,[3] all fish were between 1 and 19 years of age.

Stable carbon and nitrogen isotope measurements were performed on dorsal fin spines. Dorsal fin spines were extracted and frozen prior to laboratory processing. After thawing, fin spines were cleaned of epidermal and dermal tissue, soaked in a dilute solution of $HClO_3^-$ (bleach) to remove excess tissue, and then washed thoroughly with double-distilled water (no significant difference was observed with the dilute bleach method of cleaning and simply scraping the spine clean). Collagen was extracted according to the method of Tuross et al. (1988), who found that collagen extractions obtained with ethylenediaminetetraacetic acid (EDTA) yielded higher demineralization than those obtained with hydrochloric acid. Contamination of EDTA had been detected at less than 1 ng EDTA per mg of dry protein (Tuross et al., 1988). Spines of each individual were soaked separately in 50 mL of 0.5M EDTA, pH 7.2, at 4°C and shaken on a laboratory shaker for five days to remove mineralized bone. Mineralized bone was considered removed by evidence of a translucent, pale yellow appearance (Tuross et al., 1988). The remaining collagen was washed with dilute NaOH, rinsed thoroughly with double distilled water, and freeze-dried.

An investigation of sample preparation techniques was conducted to ensure accurate data collection. Incomplete removal of the mineral phase of the dorsal fin spine would cause erroneous ^{13}C-enriched values. In turn, poor conversion of collagen carbon to CO_2 would result in CO production and inaccurate ^{15}N-enriched values owing to $^{13}C^{16}O$, which interferes with the $^{15}N^{14}N$ signal on the mass spectrometer. These sources of contamination were most likely to occur in large samples, reflecting a relation between sample size and isotope value.

Owing to the large size of these dorsal fin spines, multiple sections were taken from all samples to ensure that the whole spine was measured isotopically. Spines were divided into approximately 3-mg sections; therefore, spines from larger mackerel had more sections than did spines from smaller mackerel. Each section of the dorsal fin spines was placed in a separate quartz tube with elemental copper and cupric oxide and sealed under vacuum. These sections were converted to CO_2 and N_2 gas with modified Dumas combustion (850°C for two hours) (Macko, 1981). The CO_2 and N_2 were then isolated cryogenically and analyzed on Finnigan MAT 251 and Nuclide 3-60-RMS isotope ratio mass spectrometers. The reproducibility of the measurements for $\delta^{13}C$ was ±0.2‰ and ±0.3‰ for N_2. Minimum sample size was 50 µg for both $\delta^{13}C$ and $\delta^{15}N$.

Stable carbon and nitrogen isotope measurements were performed on 65 and 64 dorsal fin spines, respectively. Stable isotope ratios, denoted in parts per mil, were calculated in terms of δ as follows:

[3] DeVries, D. A., and C. B. Grimes. 1991. Spatial and temporal variation in age composition and growth of king mackerel *Scomberomorus cavalla* from the southeastern U.S., 1986–1989; implications for stock structure and recruitment variability. U.S. Dep. Commer., NOAA, NMFS, 3500 Delwood Beach Rd., Panama City, FL 32408-7403. Unpubl. manuscript, 41 p.

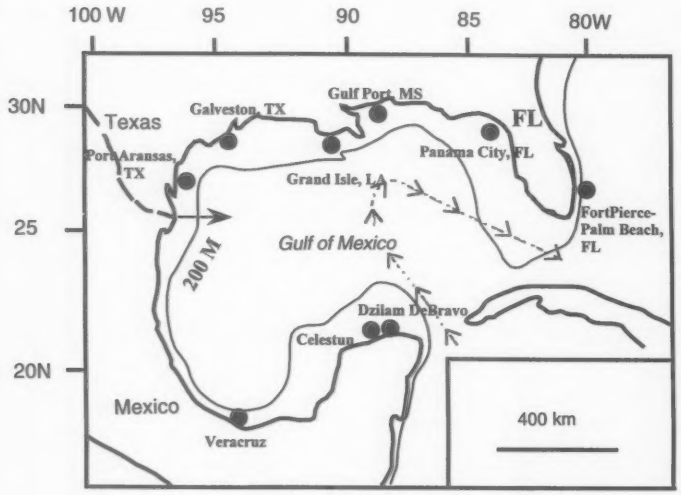

Figure 1

King mackerel study sites. Dotted line is mean position of the Loop Current. Arrow heads on the dotted line denote direction of flow. Solid arrow indicates convergence zone at Brownsville, TX.

Table 2

Mackerel collection data and range of stable isotope (C and N) values for each dorsal spine analyzed. nd = no data.

Collection site	Sex	Fork length (cm)	Month of collection	Range of $\delta^{13}C$ (‰) for each spine	Range of $\delta^{15}N$ (‰) for each spine
Celestun, Mexico	male	91	12	4.1	0.9
Celestun, Mexico	female	81	12	1.5	0.8
Celestun, Mexico	male	78	12	2.5	0.7
Celestun, Mexico	female	76	12	3.3	4.3
Gulf Port, Mississippi	male	126	7	3.0	0.2
Gulf Port, Mississippi	female	120	7	2.1	0.9
Gulf Port, Mississippi	male	100	7	2.6	0.5
Gulf Port, Mississippi	male	117	7	2.3	0.2
Gulf Port, Mississippi	male	85	7	2.9	0.5
Gulf Port, Mississippi	female	113	7	2.5	1.1
Veracruz, Mexico	male	71	5	1.7	0.7
Veracruz, Mexico	male	68	2	3.1	0.3
Dzilam DeBravo, Mexico	unknown	73	11	3.3	0.9
Dzilam DeBravo, Mexico	unknown	51	11	0.7	1.0
Dzilam DeBravo, Mexico	unknown	54	11	1.8	0.5
Dzilam DeBravo, Mexico	unknown	71	11	2.3	0.2
Dzilam DeBravo, Mexico	unknown	78	11	1.8	1.4
Dzilam DeBravo, Mexico	unknown	70	11	1.4	3.5
Dzilam DeBravo, Mexico	unknown	64	11	0.7	nd
Dzilam DeBravo, Mexico	unknown	79	11	3.5	0.2
Dzilam DeBravo, Mexico	unknown	65	11	1.4	0.2
Dzilam DeBravo, Mexico	unknown	93	11	1.3	0.3
Dzilam DeBravo, Mexico	unknown	80	11	1.2	0.4
Dzilam DeBravo, Mexico	unknown	73	11	2.4	1.1
Dzilam DeBravo, Mexico	unknown	69	11	1.3	0.2
Dzilam DeBravo, Mexico	unknown	65	11	0.9	4.1
Dzilam DeBravo, Mexico	unknown	73	11	3.8	0.5
Dzilam DeBravo, Mexico	unknown	80	11	3.1	3.0
Dzilam DeBravo, Mexico	unknown	65	11	3.6	2.8
Dzilam DeBravo, Mexico	unknown	89	11	2.4	3.2
Dzilam DeBravo, Mexico	unknown	69	11	1.2	1.8
Dzilam DeBravo, Mexico	unknown	79	11	nd	0.9
Galveston, Texas	unknown	86	7	2.7	0.6
Galveston, Texas	unknown	70	7	2.3	0.7
Galveston, Texas	unknown	67	7	2.9	0.3
Port Aransas, Texas	unknown	81	7	3.9	0.2
Port Aransas, Texas	unknown	81	7	0.4	0.8
Port Aransas, Texas	unknown	113	7	4.3	0.5
Port Aransas, Texas	unknown	94	7	3.0	2.9
Port Aransas, Texas	unknown	75	7	3.0	1.1
Port Aransas, Texas	unknown	97	7	3.4	0.7
Port Aransas, Texas	unknown	88	7	2.2	0.6
Port Aransas, Texas	unknown	91	7	1.6	0.1
Port Aransas, Texas	unknown	121	7	5.4	1.0
Grand Isle, Louisiana	unknown	88	7	2.6	0.1
Grand Isle, Louisiana	unknown	99	7	2.5	0.9
Grand Isle, Louisiana	unknown	49	7	2.4	1.1
Grand Isle, Louisiana	unknown	74	7	1.1	0.3
Grand Isle, Louisiana	unknown	58	7	0.4	0.1
Grand Isle, Louisiana	unknown	73	7	2.7	0.2
Grand Isle, Louisiana	unknown	75	7	2.4	0.2
Grand Isle, Louisiana	unknown	96	7	4.2	0.2
Grand Isle, Louisiana	unknown	61	7	1.9	0.9
Grand Isle, Louisiana	unknown	80	7	3.1	0.4
Panama City, Florida	female	85	6	2.6	0.3
Panama City, Florida	female	80	6	2.9	0.2

Table 2 (continued)

Collection site	Sex	Fork length (cm)	Month of collection	Range of $\delta^{13}C$ (‰) for each spine	Range of $\delta^{15}N$ (‰) for each spine
Panama City, Florida	female	84	6	1.5	0.6
Panama City, Florida	female	81	6	3.0	0.6
Panama City, Florida	female	80	6	2.2	0.3
Panama City, Florida	female	90	6	2.0	0.4
Fort Pierce-Palm Beach, FL	female	102	5	2.2	0.5
Fort Pierce-Palm Beach, FL	male	100	5	3.5	0.3
Fort Pierce-Palm Beach, FL	male	87	5	3.5	0.7
Fort Pierce-Palm Beach, FL	male	71	3	1.3	nd
Fort Pierce-Palm Beach, FL	male	75	3	2.1	0.4
Fort Pierce-Palm Beach, FL	male	70	3	1.7	0.2

$$\delta X\,[‰] = (R_{sample}/R_{standard} - 1) \times 10^3, \quad (1)$$

where X = the heavier isotope (either ^{13}C or ^{15}N); and
R = the ratio (either ^{13}C:^{12}C or ^{15}N:^{14}N).

The working standard for carbon was tank CO_2 which was identified as $\delta^{13}C_{PDB} = -1.85‰$, and the standard for nitrogen was N_2 from air, which is 0‰ by definition (see Eq. 1).

Because spines were too large to measure whole, they were divided into segments that were analyzed separately, and the weighted average was calculated as

$$\frac{\sum_{n=1}^{x}(W_n)(\delta_n)}{\sum_{n=1}^{x} W_n} \quad (2)$$

where W_n = weight of the segment in milligrams; and
δ_n = isotopic value for the segment.

Multivariate analysis of covariance (MANCOVA) was used to determine which independent variables had significant effects in the general linear models (GLM) (Eqs. 3 and 4) (SAS, 1990).

$$\delta^{15}N \text{ or } \delta^{13}C = collection\ site + season + sex; \quad [\text{length was used as a covariate.}] \quad (3)$$

$$\delta^{15}N \text{ or } \delta^{13}C = region + season + sex; \quad [\text{length was used as a covariate.}] \quad (4)$$

If an independent variable did not have a significant effect on the model, the variable was eliminated and the GLM was conducted again. Least squared means (LSmeans) and a pairwise comparison, with a 95% confidence interval (P=0.05), were performed to determine significant differences in nitrogen and carbon isotope data between sample collection sites and regions. As king mackerel increased in fork length, an increase in ^{15}N was observed (Fig. 2B); therefore, analysis of covariance was used to control for differences in fish size. Fork length was used as the covariate.

Results

No significant correlation between weight of the fin spine sample segment and $\delta^{15}N$ (r^2=0.03) or $\delta^{13}C$ (r^2=0.06) was detected for any of the samples, suggesting that the mineral phase had been completely removed, and 100% collagen carbon and nitrogen as CO_2 and N_2 had been recovered, respectively.

Isotopic variations within individual spines were examined to try to determine the life history of individuals. Spines were delineated into three portions (tip, mid, and base) (Fig. 3). The base of the spine is believed to contain more recently acquired material. Isotopic trends in carbon, along the length of the spine, were observed for many sites. Isotopic values for carbon became lighter as the fish aged (from tip to base); however, few trends existed for nitrogen. In general, the isotopic difference within the spine was generally less for nitrogen compared with carbon.

Nitrogen and carbon isotopic differences were observed between various sites and regions (Table 1; Fig. 4). In the pairwise comparison, more significant differences were found between individual sites for nitrogen isotope ratios than for carbon isotopic ratios (Fig. 4). Nitrogen isotopic data displayed a geographical pattern (Fig. 5). In general, king mackerel from Mississippi, Louisiana, and Texas were ^{15}N-enriched in contrast with those from the Mexican

and Florida sites. Spines of individuals from Florida were typically ^{13}C-depleted in contrast with those from the Mexico, Mississippi, Louisiana, and Texas sites.

After the nonsignificant variable (sex) was eliminated from the GLM (Eq. 3), the season of sample collection (P=0.0001), fork length (P=0.031), and collection site (P=0.0001) influenced the variation in nitrogen isotope data (F=9.27; P=0.0001 for the overall model). Only collection site (P=0.028) significantly influenced the revised GLM (Eq. 3) for $\delta^{13}C$ (F=2.38; P=0.0281 for the overall model). A GLM was also constructed for the three regions in this study: Florida, Mexico, and northwestern Gulf of Mexico (Eq. 4). These regions were determined on the basis of previous king mackerel stock structure studies (Baughman, 1941; Trent et al., 1987; Johnson et al., 1994; May[2]) and isotopic patterns observed in previous studies (Fry, 1983; Macko et al., 1984) as well as in this study. The GLM (Eq. 4) for $\delta^{15}N$ (F=26.42; P=0.0001) showed that collection site (P=0.0001) and fork length (P=0.0023) were statistically significant regionally. Collection site (P=0.023) and fork length (P=0.047) also had a significant regional influence on $\delta^{13}C$ (F=4.04; P=0.011) (Eq. 4).

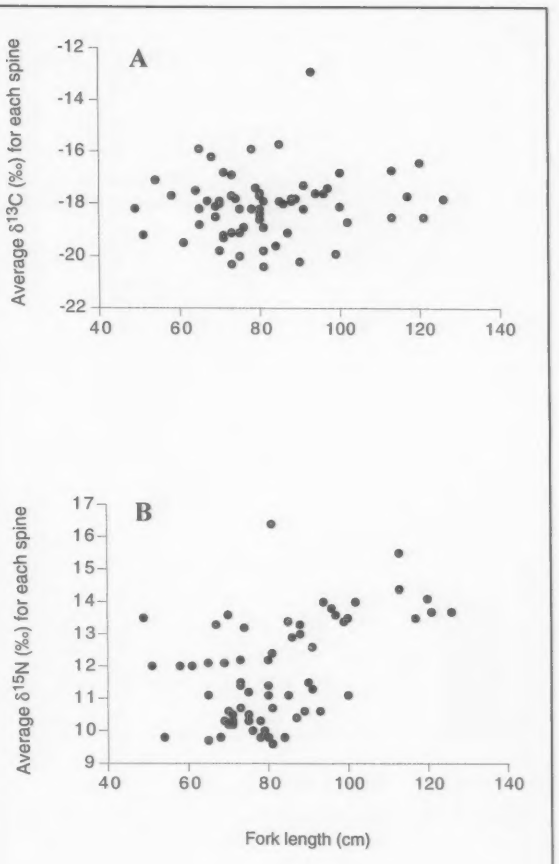

Figure 2

King mackerel fork length versus (**A**) stable carbon and (**B**) nitrogen isotopes. King mackerel fork length showed a positive relation to nitrogen isotopic values, but not to carbon isotopic values.

Discussion

Although the dorsal fin spines were divided into multiple sections, and isotopic trends were observed along a spine (Fig. 3, A and B), life history could not be determined from isotopic data because it was not possible to assign accurately an age to a particular portion of the spine. The length-at-age relation varies regionally and shows large individual variation (DeVries and Grimes[3]). For example, male king mackerel from the eastern Gulf of Mexico, with a fork length of 105–110 cm, ranged from 4 to 22 years of age (DeVries and Grimes[3]). Additionally, female king mackerel are larger than males at a given age (Beaumariage, 1973; Johnson et al., 1983; DeVries and Grimes[3]) and sex was not known for the majority of the fish analyzed (Table 2).

Isotopic differences within individual dorsal spines were studied. One would generally expect the king mackerel with the greater fork length to have a larger range of isotopic values within its dorsal spine owing to variation in trophic-level feeding with size; however, no clear trends were found (Table 2). For example one 113-cm-FL female king mackerel from Gulf Port, MS, exhibited little variation among the segments analyzed. The $\delta^{15}N$ varied by only 1.1 and the isotopic ratio of carbon varied by only 2.5‰. Conversely, a 76-cm-FL female from Celestun, Mexico differed by 4.3‰ in nitrogen and 3.3‰ in carbon among the spine segments analyzed.

Additionally, an isotopic trend of an individual spine becoming heavier over time (from tip to base) would be expected because of an increase in trophic level feeding; however, this trend was not generally observed in the sites or regions for either carbon or nitrogen (Fig. 3). A greater enrichment in nitrogen, compared with carbon, would be expected for an increase in trophic level feeding (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981); however this tendency was not observed. In fact, carbon isotopic trends were contradictory to this assumption, and no clear nitrogen isotopic trends could be detected

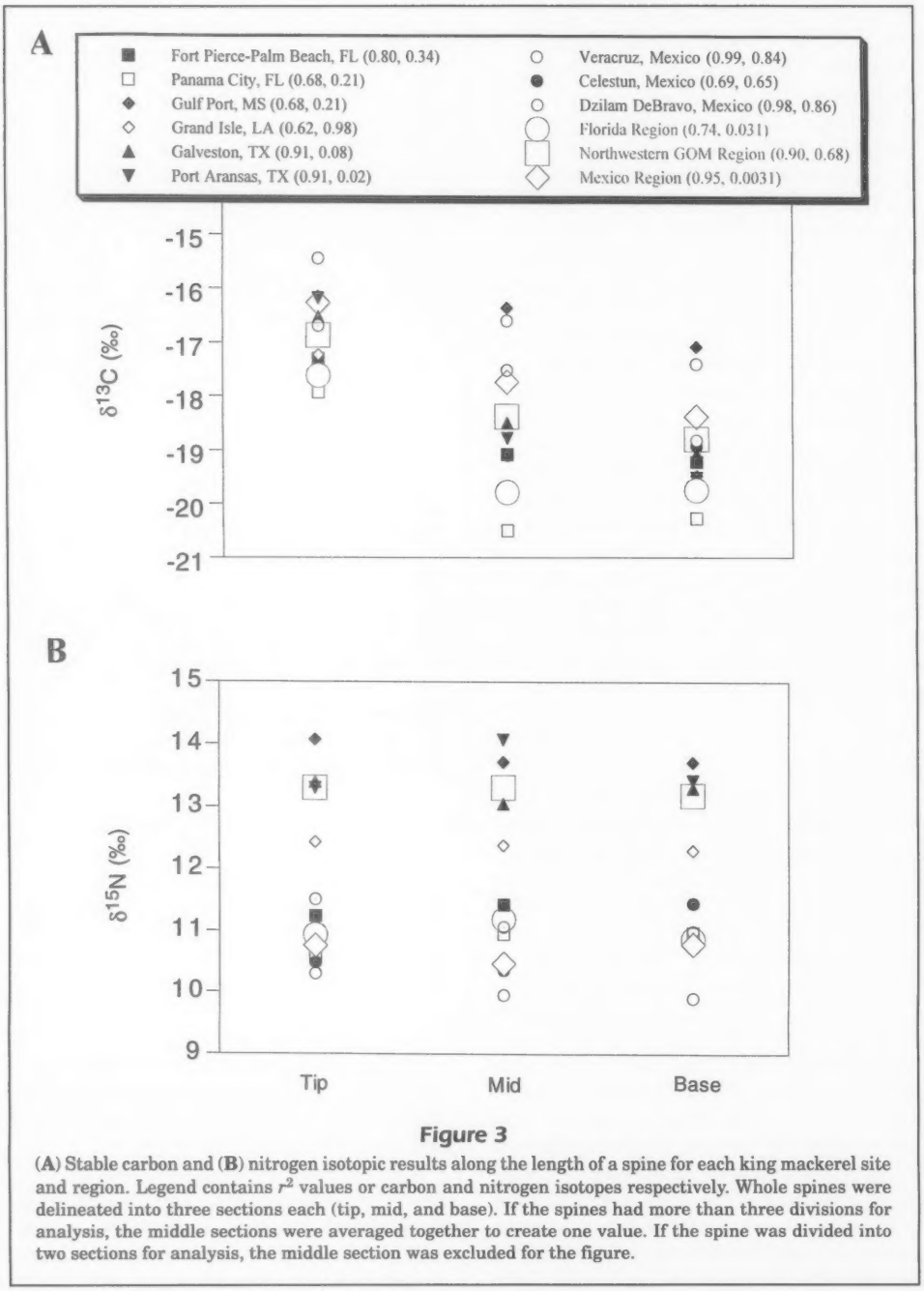

Figure 3

(**A**) Stable carbon and (**B**) nitrogen isotopic results along the length of a spine for each king mackerel site and region. Legend contains r^2 values or carbon and nitrogen isotopes respectively. Whole spines were delineated into three sections each (tip, mid, and base). If the spines had more than three divisions for analysis, the middle sections were averaged together to create one value. If the spine was divided into two sections for analysis, the middle section was excluded for the figure.

along the individual spines for the sites or regions. The data suggest a factor other than change in trophic level determines isotopic values found within the spines. Numerous factors could influence the range and trend of isotopic values found within a dorsal spine, such as feeding region, trophic-level status of the individual, and the manner in which the spine was segmented.

Stable carbon and nitrogen isotopic values at the base of the food chain vary within the Gulf of Mexico (Table 1), particularly among waters off southern Florida and the northwestern Gulf of Mexico. We

observed similar differences for mean $\delta^{15}N$ values of king mackerel spines collected in these regions. The $\delta^{15}N$ data of Macko et al. (1984), Fry (1983), and this study all showed an ^{15}N-enriched in the northwestern Gulf of Mexico relative to samples collected in Florida and Mexico. Enriched nitrogen values are often observed off the mouths of estuaries (e.g. Cifuentes et al., 1989). This enrichment often reflects the assimilation of isotopically altered inorganic nitrogen from riverine sources by algae. The influence of the Mississippi River could account for the more positive $\delta^{15}N$ values (Lopez-Veneroni[4]) detected in the northwestern Gulf of Mexico.

In contrast to the $\delta^{15}N$ data for the king mackerel, $\delta^{13}C$ measurements were not as discriminating between sites (Fig. 4) or regions (Table 1). Although not significant, the king mackerel $\delta^{13}C$ values for the northwestern Gulf of Mexico region were more negative than those for the Mexico region. More negative $\delta^{13}C$ values were also detected at the base of the food chain in the northwestern Gulf of Mexico region in comparison with those for Florida (Table 1). The influence of the Mississippi River on the northwestern Gulf of Mexico area is most likely the primary reason for $\delta^{13}C$ values being more negative. Although CO_2 depletion resulting from enhanced primary production can increase $\delta^{13}C$ values (Raven et al., 1993), the primary impact of the Mississippi River is the large terrestrial input of particulate organic matter (Trefry et al., 1994) leading to more negative $\delta^{13}C$ values.

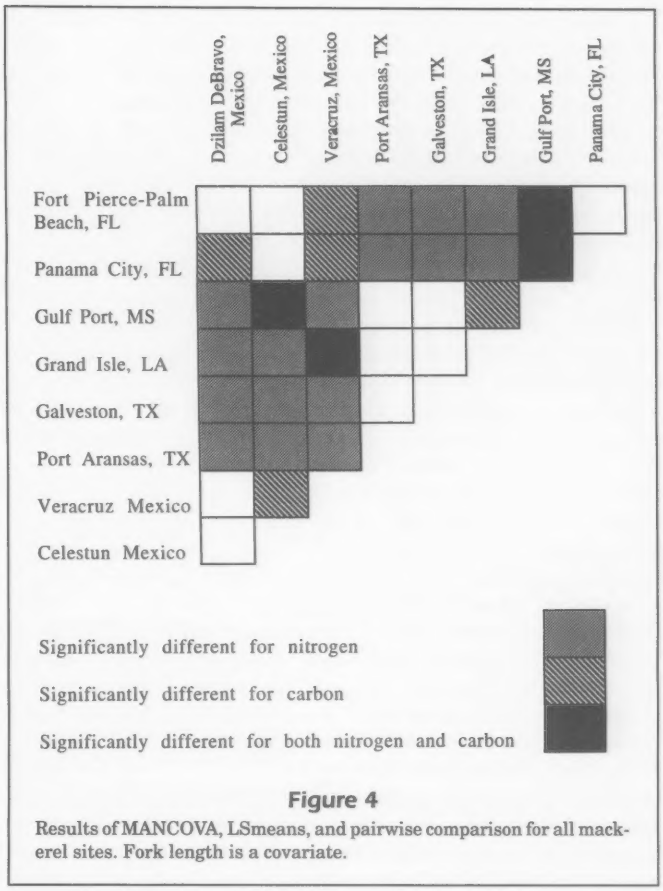

Figure 4

Results of MANCOVA, LSmeans, and pairwise comparison for all mackerel sites. Fork length is a covariate.

Commonly, less variability is observed in carbon isotopes than with nitrogen. This trend was not observed in this study. Our results, however, are consistent with some previous studies that reported that $\delta^{15}N$ data could be more discriminating than $\delta^{13}C$ data. For example, Sholto-Douglas et al. (1991) used carbon and nitrogen isotopes to study food web relations among plankton and pelagic fish and found greater variability in $\delta^{13}C$ data than in $\delta^{15}N$ measurements. Perhaps these systems have numerous carbon sources that create greater than expected variation in stable carbon isotope values, thereby rendering them ineffective.

Numerous studies have observed seasonal migrations of king mackerel. King mackerel migrate along the eastern coast of the Gulf of Mexico and into the northern Gulf of Mexico from southeastern Florida (wintering grounds) in the summer (Trent et al., 1987; Sutter et al., 1991; see also Johnson et al., 1994). Migrations may extend as far as Galveston and Port Aransas, TX (Williams and Sutherland, 1978). A return migration from the northern Gulf of Mexico into southeast Florida occurs in late summer and early fall (Williams and Sutherland, 1978). While the king mackerel that winter in southeast Florida are migrating into the northern Gulf of Mexico, a simultaneous migration from the Yucatan area (wintering grounds) occurs along the western coast of the Gulf of Mexico into the northern Gulf of Mexico (Trent et al., 1987; see also Johnson et al., 1994).

Wind circulation along the Mexican and south Texas coast during the late spring and early summer may cause upwelling off the Texas-Mexico border (Dagg et al., 1991). Consequently, coastal bound-

[4] Lopez-Veneroni, D. 1997. Oceanography Department, Texas A&M University, College Station, TX 77843. Manuscript in prep.

ary water masses off Mexico and south Texas may collide and form a convergence zone that directs low-salinity waters offshore, near Brownsville, TX (Vastano[5]) (Fig. 1). This convergence zone may act as a temporary boundary between northwestern Gulf of Mexico fish and Mexico fish.

The innate migratory patterns of the king mackerel can influence the isotopic values observed in their dorsal spines. The location in which food is assimilated should directly influence the isotopic value recorded in the spine. Consequently, the area in which the individual fed, rather than collection site of the individual, would be detected in the spine. Therefore, determination of groups of king mackerel from collection site alone, may be inappropriate. In addition, regional groupings of the mackerel that were based upon isotopic data and on previous king mackerel studies may be more suitable for drawing conclusions.

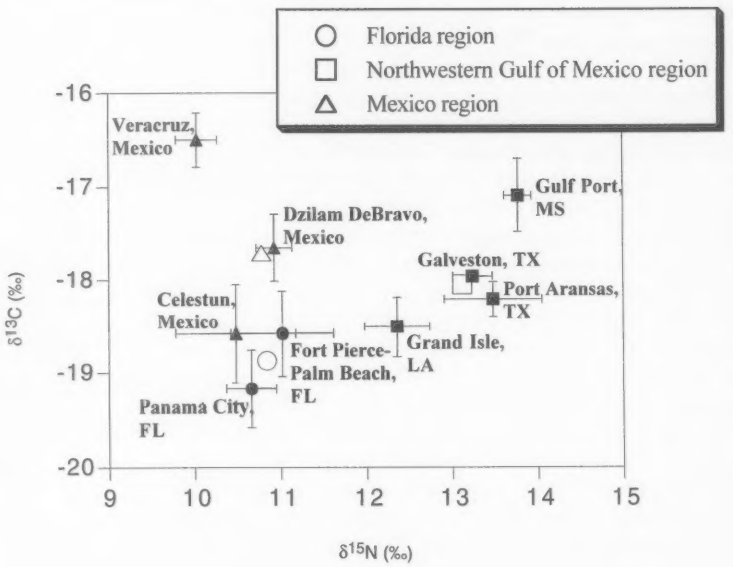

Figure 5

Weighted mean $\delta^{15}N$ versus $\delta^{13}C$ values for all king mackerel sample collection sites and regions. Number of samples per site can be seen in Table 2. Standard error bars are shown for sites. Regions were defined according to stable isotopic data and stock structure studies (Baughman, 1941; Fry, 1983; Macko et al., 1984; Trent et al., 1987; Fable et al., 1990; Johnson et al., 1994; May[2]).

Likewise, the migratory nature of king mackerel complicates the use of season of collection as a variable (Table 2). For example, the GLM (Eq. 3) indicated that the season in which the specimens were collected, fork length, and collection site influenced the nitrogen isotope results. However, when the data were divided into regions, fork length and region were the only variables that had a significant effect on the GLM (Eq. 4). Again, consideration of the data by region, as opposed to individual site, may be more appropriate, particularly because, in the former case, time of collection did not bias the isotopic findings.

Our method of using stable isotopes is a new approach in trying to determine the number and location of king mackerel groups. An advantage of isotopic analysis over that of genetics is that stable isotopes enable researchers to view significant changes in an individual, whereas genetic methods require generations to see significant variations. The disadvantage to stable isotopes is that the signal is acquired only from areas in which food is assimilated, which may or may not represent the location and number of king mackerel groups. Although the number and location of king mackerel stocks have been researched previously by using genetic techniques (see below), several scenarios exist. Research by DeVries and Grimes (1991) has suggested the possibility of three stocks: a western Gulf of Mexico, an eastern Gulf of Mexico, and an Atlantic stock. From mitochondrial DNA data, Gold et al. (in press) found weak genetic differences between Atlantic and Gulf of Mexico king mackerel that implied more than one stock. Additionally, Johnson et al. (1994), using electrophoretic data, suggested the existence of two stocks, eastern and western, within the Gulf of Mexico. The idea of separate eastern and western stocks of king mackerel within the Gulf of Mexico has also been supported by Baughman (1941), May,[2] and Trent et al. (1987) with observational, electrophoretic, and catch results, respectively.

Our $\delta^{15}N$ data showed significant differences between king mackerel caught in Mexican and Florida waters in contrast to those collected in the northwestern Gulf of Mexico. Thus, our isotopic results suggest that at least two distinct groups exist within

[5] Vastano, D. 1995. Oceanography Department, Texas A&M Univ., College Station, TX 77843.

the Gulf of Mexico (Fig. 5). Statistically, the Mexico and Florida regions are significantly different in carbon isotopes; however, neither region differs significantly from the northwestern Gulf of Mexico. It is conceivable that a separate Mexico and Florida group of king mackerel exists. Possibly neither site differed significantly from the northwestern Gulf of Mexico owing to individuals from both the Mexico and Florida regions being contained in the catch from the northwestern Gulf of Mexico. Recall, the northwestern Gulf of Mexico individuals were collected in the summer when migrations to the northwestern Gulf of Mexico from Florida and Mexico have been documented (Trent et al. 1987; Sutter et al. 1991). However, the similarity in nitrogen isotopic composition indicates that the Florida and Mexico regions are related.

A year-round sustained population in the northwest Gulf of Mexico would contribute to their isotopically different nitrogen values compared with Mexican and Florida fish. Other studies have surmised that Louisiana may have a resident population (Fisher, 1980; Fable et al., 1987) along a broad area from the Mississippi delta westward to regions off Texas, which are adjacent to oil rigs (Trent et al., 1983). These artificial structures may attract bait fish (Wickham et al., 1973). Northwestern Gulf of Mexico fish, being significantly ^{15}N-enriched, might be a nonmigrating or a separate group of king mackerel that feed on an isotopically enriched food source compared with king mackerel from Mexico and Florida. Alternatively, it is conceivable that the individuals are migratory and that the isotopic signal is due to assimilation of material from the northwestern Gulf of Mexico region although they are not permanent inhabitants of the region.

Physical dynamics within the Gulf of Mexico may influence mixing between sites and therefore the isotopic values in mackerel found at different sites. The primary current in the Gulf of Mexico is the Loop Current, which enters the Gulf of Mexico through the Yucatan Channel and exits through the Florida Straits (Leipper, 1970; Cooper et al., 1990) (Fig. 1). This current is formed by waters from the western, north, and south Atlantic and the Mediterranean Sea that flow into the Caribbean Sea (Koch et al., 1991). It has a mean position of 88° and 89°W and 27°N (Auer, 1987). Although the Loop Current reaches into the northern Gulf of Mexico, its influence is to the east of the Mississippi Delta. Thus, Mexican and Florida fish could be linked by the Loop Current to the extent that they consume isotopically similar food sources. In contrast, fish in the northwestern Gulf of Mexico are most likely minimally affected by the Loop Current.

Northwestern Gulf of Mexico fish may also be strongly influenced by runoff from the Mississippi River system (Dagg et al., 1991). The majority of this runoff (two thirds) is westward and contains high concentrations of dissolved nutrients in relation to the open Gulf of Mexico (Dagg et al., 1991). Dagg et al. (1991) also suggested that the Mississippi River system is the ultimate source of much of the biological productivity on the Louisiana and Texas shelf. The flow of the Mississippi River into the northwestern area influences the isotopic differences within these Gulf of Mexico sites (Lopez-Veneroni[4]). Although Gulf Port, MS, is east of the Mississippi river, specimens collected from this area could conceivably be feeding in or near the Mississippi River Plume region. Discharge from the Mississippi River is transported west along the shore (Dagg et al., 1991), and consumption of prey from this region would be heavily influenced by the Mississippi River leading to ^{15}N-enriched values found in this study. Furthermore, Dagg et al. (1991) stated that king mackerel from the northern Gulf of Mexico generally consumed prey that were estuarine dependent and are, therefore, most likely influenced by runoff.

Conclusions

Stable nitrogen isotope values of spines of king mackerel varied geographically. The northwestern Gulf of Mexico (+13.1‰) was isotopically distinct from the Mexican and Florida (+10.8‰ and +10.8‰) regions. We interpret these results to mean that there are, at least, two distinct groups of king mackerel within the Gulf of Mexico. Our results contrast with certain previous stock-structure assessments that distinguish only between Gulf of Mexico and Atlantic stocks. Stable carbon isotopes were able to distinguish between Mexico and Florida regions, although, not the northwestern Gulf of Mexico region. Although carbon isotopes were expected to be less variable than nitrogen, owing to the enrichment from trophic level to trophic level, they were found to be more variable within individual spines. The variability and perplexing isotopic trends within individual spines create difficulties in drawing conclusions from the data for stable carbon isotopes. In addition, fewer significant differences were detected between sites for stable carbon isotopes than for nitrogen isotopes. Stable carbon isotopes may be more useful when the isotopic discrimination among food resources is greater, which may be found when individuals also feed in coastal habitats. King mackerel, being of great commercial and recreational value, need to be managed with a clearer understanding of the number of groups

that exist. The isotopic data we have generated in conjunction with genetic research and tagging studies may be able to answer questions pertaining to location and number of king mackerel groups.

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Effectiveness of four industry-developed bycatch reduction devices in Louisiana's inshore waters

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Abstract.—Trawling was conducted in three areas of coastal Louisiana during the two inshore shrimp seasons of 1992 to evaluate the effectiveness of four industry-developed bycatch reduction devices (BRD's). Each BRD (Authement-Ledet excluder, Cameron shooter, Lake Arthur excluder, and Eymard accelerator) was towed alongside a control net 72 times; tows were equally divided between areas and seasons. The Authement-Ledet excluder, Cameron shooter, and Lake Arthur excluder BRD's caught fewer fish (–36%, –51%, and –21%, respectively), but also fewer shrimp (–18%, –16%, and –24%) than corresponding control nets. Biomass catch differences were –42%, –33%, and –21% for fish and –14%, –14%, and –17% for shrimp. The Eymard accelerator caught 26% more fish numerically, 19% less fish biomass, and more shrimp (38% in numbers, 26% in biomass) than control nets. Differences between catches obtained with BRD nets and those with control nets depended upon the organisms present in an area. Abundances and size distributions of many species differed between areas; thus BRD's may have to be selected for the area where they are intended to be used.

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The inshore shrimping area of Louisiana is typically the waters landward of the barrier islands and the general Gulf of Mexico shoreline. The Louisiana inshore shrimp fishery is managed as three geographic zones (Fig. 1) and has two inshore shrimping seasons. Brown shrimp, *Penaeus aztecus*, dominate spring catches, whereas white shrimp, *P. setiferus*, dominate fall catches, although both species are caught during each season. In 1992, nearly 101 million kg of shrimp, valued at about $389 million, were landed commercially in the Gulf of Mexico (National Marine Fisheries Service, 1993). From 1986 to 1989, 40% of the total commercial catch in Louisiana was caught inshore (Baron-Mounce et al.[1]).

Although fishing gears and areas fished have varied, a survey in 1987 (Keithly and Baron-Mounce[2]) characterized Louisiana's commercial inshore shrimpers as follows. The average vessel size was 10.2 m for full-time shrimpers, 6.1 m for part-time shrimpers. Smaller boats tended to be constructed of fiberglass and powered by outboard motors. About 75% to 80% of the commercial inshore shrimpers participated in the fishery on a part-time basis. State law limited the size of each trawl to a headrope of 7.6 m length when two trawls were towed in inshore waters, except for Breton and Chandaleur Sounds. The inshore shrimp fleet was not highly mobile between management zones; only about 10% of the full-time shrimpers with boats in the 20–30 ft range and 2% of the part-time shrimpers fished in more than one zone during either season. The estimated inshore shrimping effort in 1987 by management zone was 18% (zone 1), 73% (zone 2), and 9% (zone 3)(Keithly and Baron-Mounce[2]).

The otter trawl has been the primary gear used by the inshore shrimp fishery in Louisiana (Keithly and Baron-Mounce[2]), although butterfly (wing) nets, cast nets, and skimmer (bay sweepers) nets have

[1] Baron-Mounce, E., W. Keithly, and K. J. Roberts. 1991. Shrimp facts. La. Sea Grant Coll. Prog., Communications Office, Louisiana State Univ., Baton Rouge, LA, 22 p.

[2] Keithly, W. R., Jr., and E. Baron-Mounce. 1990. An economic assessment of the Louisiana shrimp fishery. Final report to NMFS NA88WC-H-MF179. Coastal Fisheries Institute, Louisiana State Univ., Baton Rouge, LA, 129 p.

Figure 1

Map of the Louisiana coastline with locations of the three study areas and the three shrimp management zones (east-west zone boundaries are denoted by dark lines).

also been used. The minimum legal stretch mesh size at the time of the present study was 3.2 cm; however, shrimpers often use larger mesh to reduce the catch of small shrimp and nontargeted (bycatch) organisms.

Some methods that shrimpers have used to reduce bycatch have included relocating to areas of lower fish concentrations, cutting openings in nets, reducing tow speeds before haulback, and modifying nets in various ways. Heightened pressure by environmental organizations and pending legislation to reduce bycatch has furthered the development of shrimp trawls equipped with bycatch reduction devices (BRD's) to reduce the catch of nontargeted organisms. Previous research on BRD designs tested in the United States has been summarized by Watson and Taylor.[3]

Some of the BRD designs used successfully in other shrimp fisheries have proven ineffective in Gulf of Mexico waters. For example, a horizontal separator panel yielded a 75% reduction in bycatch but lost 30% of the shrimp (Seidel, 1975). Seidel (1975) tested six modifications of the Pacific Northwest shrimp separator trawl, which has a vertical separator panel and several chutes for fish escapement. Shrimp losses ranged from 9.1% to 63.5%, and fish reduction ranged from 37% to 83.5%; however, the modification with the best fish reduction had a shrimp loss of 63.5%. The lowest attainable shrimp loss (6%) from a trawl with vertical separator panels of varying mesh had a 45% bycatch reduction (Watson and McVea, 1977).

The Gulf has a high diversity of bycatch species, many of which are similar in size to shrimp; shrimp, however, may represent as little as 10% of the total catch (Seidel, 1975). Prior to this study, most evaluations of BRD's in the Gulf had been conducted in offshore waters. Inshore organisms are often smaller than those caught offshore, inshore trawls and vessels are typically smaller, and trawling conditions, such as water depth and turbidity, may differ. Because of these differences, the present study was designed to determine the performance of four BRD's in inshore waters of Louisiana.

Materials and methods

Bycatch reduction devices

To gather regional expertise on trawling and BRD design, an advisory committee of shrimpers, net makers, and fishery-related agency personnel was

[3] Watson, J. W., and C. W. Taylor. 1990. Research on selective shrimp trawl designs for penaeid shrimp in the United States: a review of selective shrimp trawl research in the United States since 1973. Proceedings ASMFC Fisheries Conservation Engineering Workshop, Narragansett, RI, April 1990, 21 p.

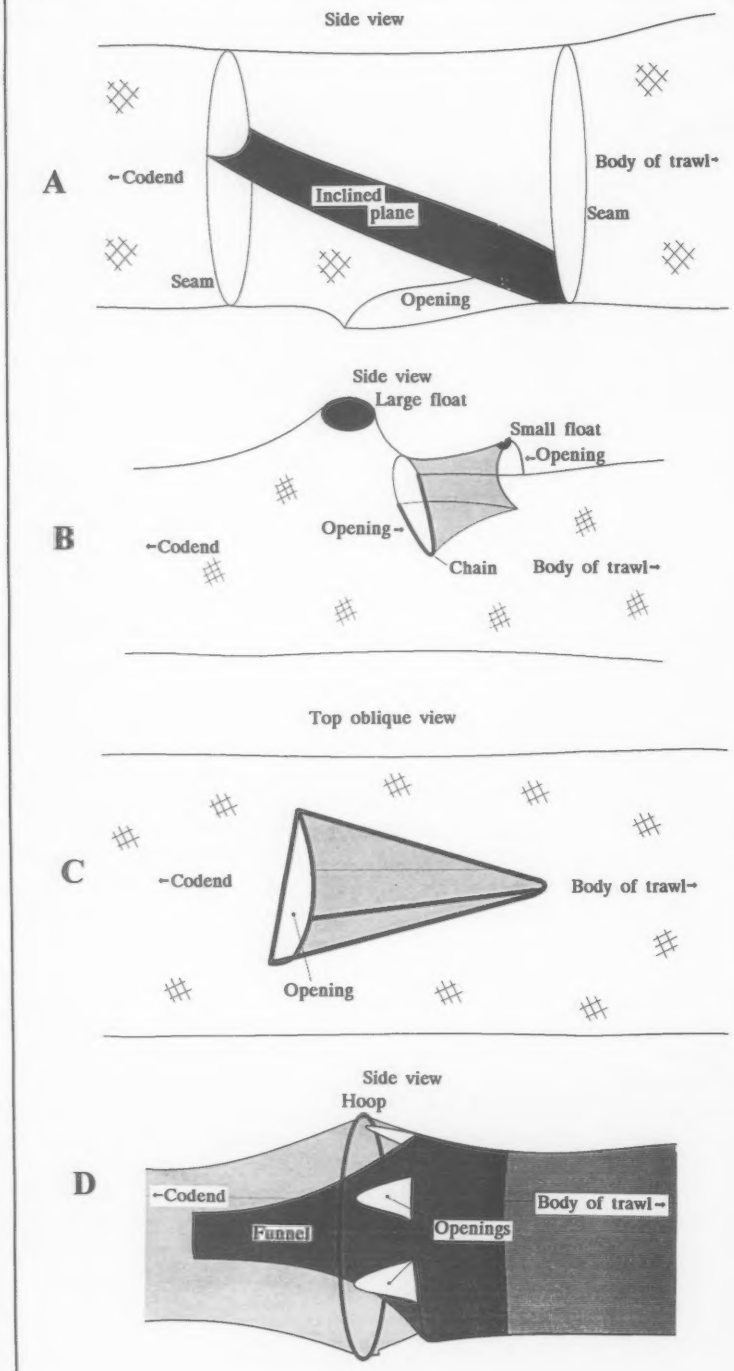

Figure 2

Diagrams of the bycatch reduction devices used in this study: **(A)** Authement-Ledet excluder; **(B)** Lake Arthur excluder; **(C)** Cameron shooter; and **(D)** Eymard accelerator.

organized. The industry committee members recommended basic trawl specifications and sampling areas. BRD's were selected from a pool of 14 industry- and NMFS-developed BRD and turtle excluder device (TED) designs suggested by members of the committee. Four industry-developed BRD designs were selected: Authement-Ledet excluder, Lake Arthur excluder, Cameron shooter, and Eymard accelerator (Fig. 2). The Cameron shooter, and very similar designs, such as the fisheye and Florida fish excluders, have had the widest use among commercial shrimpers along the U.S. Gulf of Mexico and Atlantic coasts. The other devices have been used on a limited basis in inshore and offshore waters of Louisiana, primarily in certain management zones: Eymard (zone 1), Authement-Ledet (zone 2), and Lake Arthur (zone 3).

Seven identical four-seam, nylon semiballoon trawls with 6.1-m headrope length were constructed; three were used as control nets and four were randomly selected for BRD installation. Each net had 3.5-cm stretch mesh in the body (no. 7 twine) and in the codend (no. 15 twine).

The Authement-Ledet excluder (Fig. 2A), constructed of 3.5-cm stretch mesh polyethylene webbing, was 35 meshes long and contained an inclined plane that was angled 20° from the net bottom to guide the catch upwards. The inclined plane was 18 meshes wide at the front and 30 meshes wide at the back; the back of the inclined plane was attached 18 meshes from the top seam of the trawl net. Fishes swimming forward from the codend were guided by the inclined plane to exit through the 18-mesh-wide, 40-mesh-long bottom opening.

The Lake Arthur excluder (Fig. 2B) was constructed by cutting 22 meshes across the top of the trawl

net beginning 30 meshes from the tail. A 3-mm chain was attached to the forward edge of this opening and a 10-mesh × 12-mesh long cover was attached to the rear edge of this opening. Half of a 3.8 × 7.6 cm float ("small float" in Fig. 2B) was attached under, and a 4.5-mm rope was threaded along, the forward edge of this cover. A 13 × 28 cm conical float ("large float" in Fig. 2B) was attached 10 meshes behind the opening. The chain and floats created the escape opening.

The Cameron shooter (Fig. 2C) was a 30-cm wide, 45-cm long, 15-cm deep half cone of 12.7-mm aluminum round stock. A 30-mesh opening was cut in the top of the trawl net, and the forward edge of the cone was inserted into the codend, 20 meshes back from the body of the trawl. The semicircular frame opening faced the codend and protruded inside the trawl net.

The Eymard accelerator (Fig. 2D) had a polyethylene-webbing accelerator funnel, 45 meshes in diameter and 24 meshes long surrounded by six 10 × 10 × 10 mesh triangular openings. A 60-cm hoop of rubber coated cable was attached to pull the trawl net away from the funnel after initial dive tests indicated that the funnel blocked the escape openings.

Personnel from the National Marine Fisheries Service (NMFS) Harvesting Systems Branch examined the trawls several times by using scuba equipment, and adjustments were made to the trawl rigging and the BRD's. Dye was injected into various parts of the nets around the devices to observe water flow, and the behavior of escaping fish was documented.

Sampling

A 6.7-m Boston whaler, powered by twin 115-hp outboard motors and equipped with a single-drum winch and double boom, was used to tow twin trawls off the stern (Harrington et al., 1988); one trawl contained a BRD, the other a bare control net. The nets were equipped with tickler chains, connected to an aluminum dummy door, and spread by two 0.6 × 1.07 m pinewood trawl doors. Although twin trawls are not typically towed behind commercial vessels in Louisiana, the use of twin trawls to replace single large trawls off outriggers is increasing, particularly in the Gulf of Mexico (Watson et al., 1984). Smaller inshore vessels in Louisiana, without outriggers, typically tow a single larger net behind the boat. Our twin-trawl rigging configuration was approved by the committee to ensure that the nets sampled an area as closely as possible, given the patchy nature of many species. Nets were towed for 20 minutes (time at towing speed); the speed over ground was maintained between 2.0 and 2.5 knots (2.2 kn, average) by using a Global Positioning System, as recommended by the committee. Tows were made during daylight hours, near commercial shrimp boats whenever possible. Average water depth and the salinity were recorded for each trawl tow.

The four BRD's were evaluated in each inshore shrimp zone (Fig. 1). Eighteen two-day sampling trips were made, three trips to each area during each season. Each BRD net was towed 72 times over the year. Tows were divided among the areas and seasons. The towing order and trawl side for each BRD were initially selected randomly, although the same nets were not used on consecutive tows owing to the time taken to empty the nets. The three control nets were numbered and alternated to ensure equal pairing with a particular BRD net throughout the study. Sampling was conducted during the 1992 inshore shrimp seasons, although the short spring season necessitated sampling the week before the season opened in zone 1 and a few days after the season in zone 3. This schedule was approved by the advisory committee.

Samples were tagged and placed in mesh bags in ice and water. In the laboratory, organisms in each sample were identified, counted, and the biomass of each species in a sample was weighed to the nearest 0.1 g. When numerous, individuals of a species within a sample were subsampled and the total number estimated by weight. Standard lengths of most fishes, carapace widths of crabs, and total lengths of penaeid shrimp were measured. Organisms were measured in 5-mm length increments, designated by the lower end of the length range (e.g. 10-mm class=10.0 to 14.9 mm).

Statistical analysis

Residuals were examined for univariate normality and homogeneity of variances prior to accepting the analysis of variance model. Normality was tested with the Wilk-Shapiro test, and a modified Levene test was used to test for homogeneity of variances. These tests indicated that the raw data were not distributed normally and variances were not homogeneous. The transformation ln(catch+1) was used to create a new variable that met the criteria of being approximately normally distributed with homogeneous variances. This transformed variable was used in the analysis of variance (ANOVA). Statistical analysis was performed by using the Statistical Analysis System (SAS).

Control nets The transformed catch (both numbers and biomass of abundant species) of the three control nets was used as the dependent variable in an ANOVA with season, area, and season-by-area terms

and with the interaction of these terms with the control net number, with tow as the experimental unit. Length-frequency distributions of abundant organisms collected by the control nets in each area were visually examined.

BRD's versus control nets Catches of shrimp, fish, and the nine most abundant species were analyzed. An ANOVA model was used to compare the difference between control and BRD net catches between areas and seasons. ANOVA was also used to detect differences caused by towing a BRD on the port or starboard side of the twin trawl.

The number of individuals and biomass of each species (or group) caught in a BRD net was compared with the number caught by the control net by using a univariate paired t-test. The univariate procedure is appropriate if one can assume that the probability of one species being retained within the net is independent of another species being retained. The short tow duration contributes to the chance that this assumption is valid. Paired t-tests were conducted on untransformed and log-transformed differences between BRD- and control-net catches. Percent catch differences of untransformed data were calculated to compare device nets:

$$\textit{Percentage catch difference} = \frac{\textit{Device net} - \textit{Control net}}{\textit{Control net}} \times 100.$$

Percent catch difference values could range from –100 to infinity.

Differences between areas and seasons

A univariate paired t-test was also used to compare differences between a BRD net and the control net within each area. This test had less power because the sample size was reduced by two-thirds and because the test was not able to detect as small a difference as the test with the areas combined. A similar analysis was conducted to examine device performance in each season.

For all analyses, differences between means with an alpha of 0.05 or less were considered significant. However, the exact probabilities are presented in the tables.

Results

Control nets

The control nets collected 88 species of fishes and invertebrates; fewer species were collected in the spring than in the fall in all areas (Table 1). More than 64% of the 84,919 organisms collected in the control nets were caught during the spring. Nine species represented nearly 89% of the total control-net catch. Bay anchovy, white shrimp, and hardhead catfish catches were higher in the fall, but the other

Table 1

Numbers of most abundant organisms collected in the control nets in inshore waters of Louisiana during the spring and fall of 1992.

		Spring				Fall				
		Area				Area				
Species		Borgne	Barre	Calcasieu	Total	Borgne	Barre	Calcasieu	Total	Combined total
Brown shrimp	*Penaeus aztecus*	2,842	5,375	10,593	18,810	400	481	245	1,126	19,936
Atlantic croaker	*Micropogonias undulatus*	597	8,346	5,162	14,105	240	207	2,015	2,462	16,567
Bay anchovy	*Anchoa mitchilli*	712	976	1,106	2,794	1,011	2,387	2,166	5,564	8,358
White shrimp	*Penaeus setiferus*	47	51	732	830	1,954	3,425	1,752	7,131	7,961
Hardhead catfish	*Arius felis*	423	363	2,638	3,424	1,087	1,432	1,864	4,383	7,807
Spot	*Leiostomus xanthurus*	846	2,059	1,603	4,508	180	161	339	680	5,188
Sand seatrout	*Cynoscion arenarius*	353	196	2,097	2,646	153	689	408	1,250	3,896
Blue crab	*Callinectes sapidus*	149	1,610	154	1,913	111	907	13	1,031	2,944
Gulf menhaden	*Brevoortia patronus*	86	278	1,533	1,897	54	68	623	745	2,642
Other species		273	2,108	1,152	3,533	1,263	2,273	2,551	6,087	9,620
Total		6,328	21,362	26,770	54,460	6,453	12,030	11,976	30,459	84,919
Number of species		38	51	53	66	47	65	57	82	88

six species were much more abundant in the spring. The catches from Lake Borgne were typically much smaller than catches from the other areas. Brown shrimp, hardhead catfish, sand seatrout, and gulf menhaden were most abundant in Calcasieu Lake, whereas Atlantic croaker and blue crab were most abundant in Lake Barre. Penaeid shrimp constituted 36% of the catch in the spring and 27% of the catch in the fall.

The numbers of organisms collected by the three control nets did not differ significantly. Control-net catches did not differ significantly with respect to the excluder with which they were paired. Length-frequency distributions of the abundant species differed between areas (Fig. 3).

The side of the trawl on which the control or BRD net was towed did not significantly affect catches of the abundant species. Each BRD was towed equally on each side of the twin trawl.

BRD's versus control nets

Fish All BRD nets had significantly different catches of fish from those of the control nets. Numerically, the Cameron BRD had the highest overall reduction of fish (–51%) compared with the catch of the control nets (Table 2). The Eymard BRD caught 26% more fish than the control nets. In terms of biomass, the Authement-Ledet BRD had the highest reduction (–42%), and the Eymard BRD had a 19% lower catch than the control nets.

The Authement-Ledet, Lake Arthur, and Cameron BRD nets caught fewer fish than the control nets in all size categories (Fig. 4). The Cameron BRD, in particular, had the highest reduction of small fish (≤75 mm). The Eymard BRD caught more small fish (≤85 mm) and fewer large fish than the control net.

The Cameron BRD had the best reduction in numbers of Atlantic croaker (49%), and the Authement-Ledet the best reduction in biomass (39%) (Table 3). The Authement-Ledet and Eymard BRD's caught 50% or fewer spot in terms of numbers and biomass; in contrast, the Cameron had very poor reductions for spot. Both the Cameron and Authement-Ledet BRD's caught 50% or fewer hardhead catfish than the control nets. The Cameron BRD caught 75% fewer bay anchovy than the control net, and the Authement-Ledet and Lake Arthur reduced bay anchovy by 37%. For most bycatch species, the Eymard BRD caught more than the control nets, although catches of the bay anchovy were markedly higher (83% numbers, 86% biomass).

Shrimp The catch of shrimp with all BRD nets differed significantly from the control net catch. The Cameron, Authement-Ledet, and Lake Arthur BRD's caught fewer shrimp than the control nets; shrimp catch with the Eymard was higher (38% numbers, 25% biomass) (Table 2). Numerically, the Cameron BRD had 16% fewer shrimp, and both the Cameron and Authement-Ledet had 14% lower shrimp biomass than the control nets.

Most of the catch difference between the BRD nets and corresponding control nets appeared to be smaller (≤85–90 mm) shrimp (Fig. 5). Catch differ-

Table 2

Comparison of numbers and biomass of fish and shrimp collected in bycatch reduction nets and corresponding control nets in selected inshore waters of Louisiana in 1992. SD is the standard deviation of the difference. Significance levels are 0.01 (**) and 0.05 (*). n=72. Superscripted letters denote significance levels of paired t-tests on log-transformed data: 0.01 ([a]). BRD = bycatch reduction device.

Type of catch and BRD	Numbers					Biomass (g)				
	Mean catch/tow		Percent catch difference	SD	$P>t$-value	Mean catch/tow		Percent catch difference	SD	$P>t$-value
	Control	Device				Control	Device			
Fish										
Authement-Ledet	170.5	109.0	–36	102.6	0.01**[a]	2,541.8	1,464.1	–42	1,363.1	0.01**[a]
Lake Arthur	171.4	134.7	–21	124.9	0.01**[a]	2,904.4	2,282.6	–21	1,616.5	0.01**[a]
Cameron	181.7	88.3	–51	109.8	0.01**[a]	3,068.5	2,068.1	–33	1,233.3	0.01**[a]
Eymard	190.4	239.7	26	167.6	0.01**[a]	2,980.3	2,406.3	–19	2,059.8	0.02*[a]
Shrimp										
Authement-Ledet	84.8	69.3	–18	58.1	0.03*[a]	483.1	417.5	–14	240.7	0.02*
Lake Arthur	93.2	70.9	–24	57.5	0.01**[a]	514.0	425.0	–17	218.1	0.01**[a]
Cameron	110.9	93.0	–16	77.7	0.05*[a]	579.3	500.3	–14	220.5	0.01**[a]
Eymard	98.9	136.4	38	97.4	0.01**[a]	517.2	645.3	25	340.1	0.01**[a]

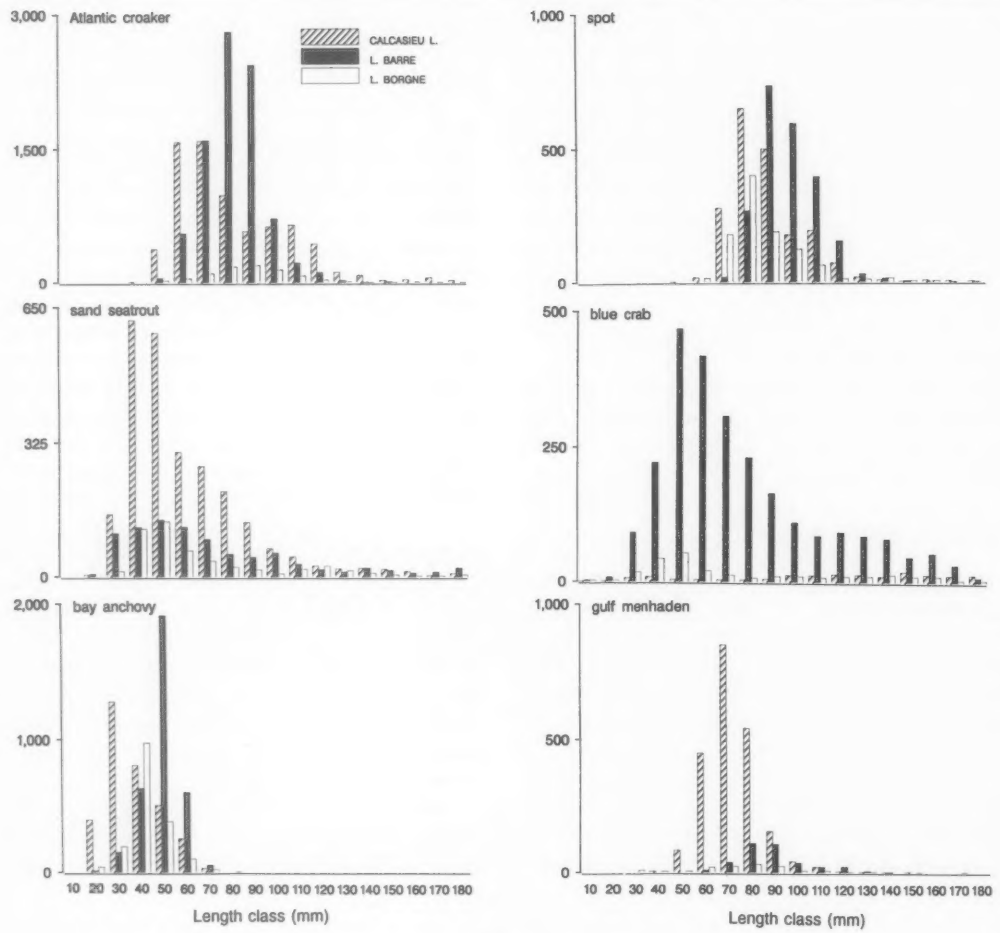

Figure 3

Length-frequency distributions of abundant species collected in control nets for the different areas. Note that length-class data are in mm, except those for catfish, which are in cm.

ences of brown shrimp and white shrimp differed for the BRD's (Table 3); fewer brown shrimp tended to be caught with the BRD nets.

Differences between areas and seasons

Although the Authement-Ledet BRD caught 18% fewer shrimp than the control nets overall, losses in Lake Barre were low and statistically nonsignificant (Table 4). Fish reduction was consistent across the areas for this BRD. The Lake Arthur BRD had a fairly consistent reduction of shrimp and fish across all areas but had the lowest shrimp catch difference in Lake Borgne and had poorer fish reductions in Calcasieu Lake. The Cameron BRD had the highest fish reduction in Lake Borgne; however, this was accompanied by the highest shrimp loss. This BRD lost the fewest shrimp in Lake Barre. The Eymard BRD caught more shrimp than the control net in all areas and reduced fish biomass in all areas, by as much as 35% in Lake Borgne.

Mean water depths and salinities differed between Lake Borgne and the other two areas. Lake Borgne (2.7 ±0.64 m) was slightly deeper than Lake Barre (1.9 ±0.34 m) and Calcasieu Lake (1.5 ±0.27 m). Mean salinities during sampling were 9.6 ±2.9‰ (Lake Borgne), 22.5 ±2.8‰ (Lake Barre), and 20.6 ±5.2‰ (Calcasieu Lake).

There were some slight differences in BRD performance between seasons (Table 5), reflecting differ-

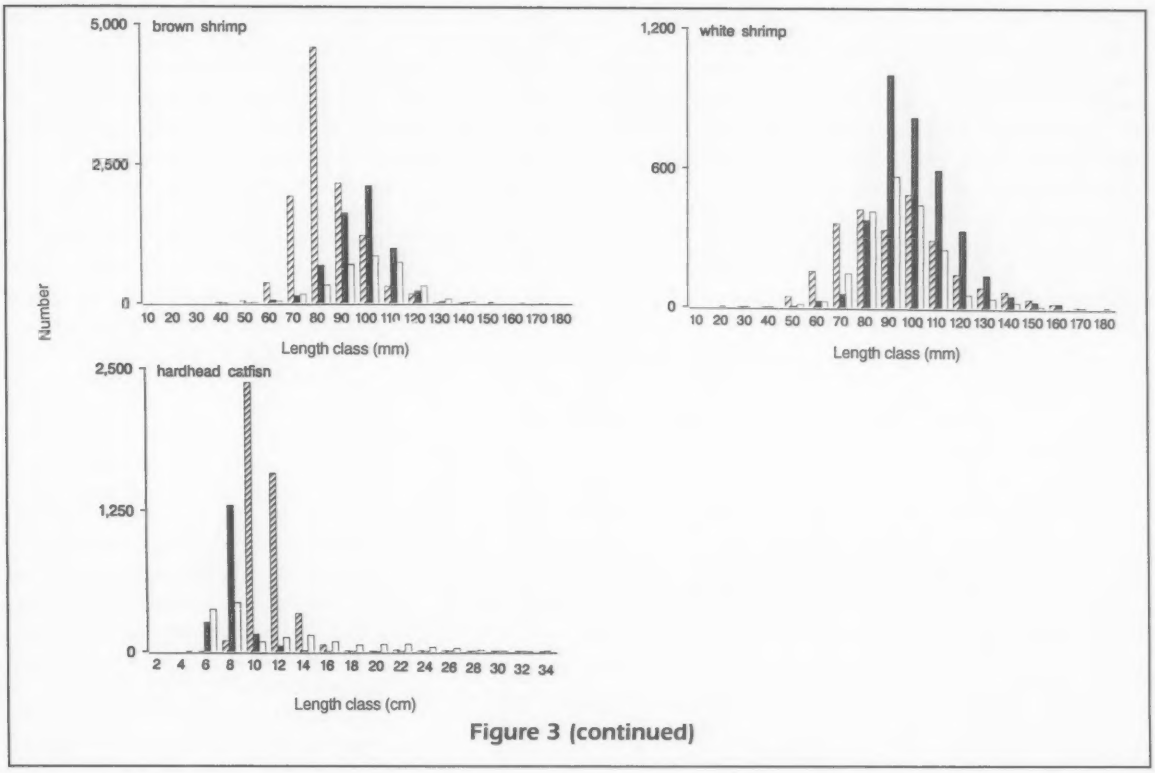

Figure 3 (continued)

ences in species composition and length-frequency distributions.

Discussion

The Authement-Ledet, Lake Arthur, and Cameron BRD's significantly reduced bycatch, but also the catch of shrimp. Excluded shrimp were primarily in the smaller size classes. The Eymard BRD caught significantly more fish and shrimp than the corresponding control nets.

The Lake Arthur and Cameron BRD's were designed with a similar opening, but the Lake Arthur BRD did not release as many fish. Because the weight of the aluminum frame of the Cameron BRD caused the device to sink slightly, the bottom of the Cameron opening was only about 15 cm from the bottom of the trawl net. In contrast, the floats of the Lake Arthur BRD raised the opening to about 30 cm above the trawl bottom. A design somewhat similar to the Lake Arthur BRD and placed 1.7 m from the end of the net did not significantly reduce bycatch in inshore waters of Alabama (Wallace and Robinson, 1994).

Reductions with the Cameron BRD (16% shrimp, 51% fish) were similar to those found by Watson et al. (1993) for the fisheye top position excluder in offshore waters (17% shrimp, 70% fish). Inshore fish are typically smaller and less able to escape by swimming; this may account for the lower reductions with the Cameron BRD. This BRD also released shrimp of most size classes; Watson and McVea (1977) found that the fish escape device, a somewhat similar device, also lost shrimp over the entire size range. Changing the location of the Cameron shooter may affect performance, although Watson et al. (1993) noted that the top position appeared to have the best effectiveness for fish reduction and shrimp retention. A bottom-mounted Florida fish shooter, placed 1.7 m from the end of a 4.9-m trawl, reduced bycatch 26% by weight and 46% by number and caught 14% fewer shrimp than an unmodified net (Wallace and Robinson, 1994). McKenna and Monaghan[4] reported that the efficiency of the Florida fish excluder depended on the size of the escape opening, placement of the excluder in the net, and the number of devices installed.

[4] McKenna, A., and J. P. Monaghan Jr. 1993. Gear development to reduce bycatch in the North Carolina trawl fisheries. Completion report to Gulf and South Atlantic Fisheries Development Foundation Cooperative Agreement No. NA90AA-H-SK052. North Carolina Div. Mar. Fish., Morehead City, NC, 79 p.

The Eymard BRD was developed to reduce the catch of larger hardhead catfish, particularly in Louisiana waters east of the Mississippi River. Hardhead catfish reductions were 6% in terms of numbers, but 51% in biomass, resulting from the loss of larger catfish. Overall, the Eymard BRD caught 26% more fish, but fish biomass was 19% less than that caught by the control net. This finding was the result of the BRD catching more fish smaller than 80 mm and fewer large fish than the control nets. The Eymard BRD caught significantly more numbers and biomass of shrimp, particularly smaller shrimp. The Eymard BRD design contained a webbing funnel, designed to carry shrimp and fish into the codend with accelerating water flow (Watson[5]). Because swimming speed of a fish is a function of size (Blaxter and Dickson, 1958), smaller fishes may not be able to swim in increased water flow, with the result that fewer shrimp and small fish can escape from the Eymard BRD than from a control net. However, dye released into the Eymard indicated that the water flow was not perceptibly increased by the funnel, probably because the funnel diameter was only slightly smaller than the net diameter. However, the Eymard BRD had a 21.6-cm greater net spread than that of the control net; this greater net opening may have resulted in the higher catches of many species. Because the nets were otherwise constructed identically, we suspect this difference was most likely due to the presence of the hoop. The polyethylene webbing may have increased the incidence of anchovy being gilled, particularly during haulback. Numerous small bay anchovies were found, upon retrieval, to be gilled in the polyethylene webbing of the Eymard BRD, and the device caught 83% more bay anchovy than the control net. In a subsequent study, a polyethylene net caught 245% more anchovies than a nylon net (Rogers et al.[6]).

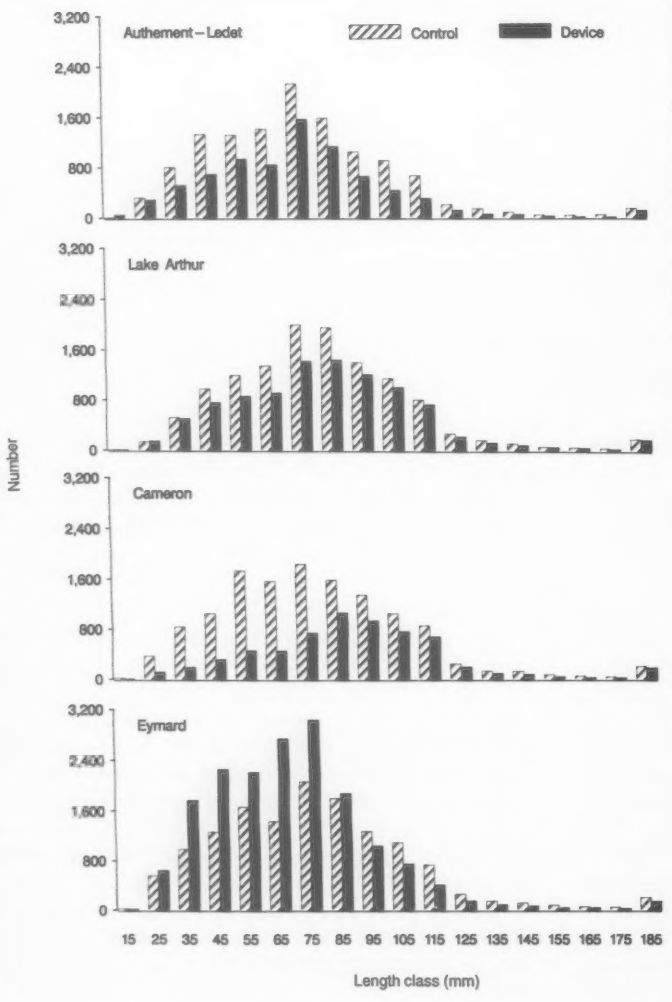

Figure 4

Length-frequency distribution of fish collected by the four devices and corresponding control nets. The 185-mm category includes all individuals greater than or equal to 180 mm.

Fish were observed escaping from several of the BRD's during diver evaluations in Florida. Divers observed several large juvenile pinfish (*Lagodon rhomboides*) escaping from the bottom openings of the Eymard BRD and numerous juvenile pinfish escaping the Authement-Ledet BRD

[5] Watson, J. W. 1988. Fish behaviour and trawl design: potential for selective trawl development. *In* S. G. Fox and J. Huntington (eds.), Proceedings of the world symposium on fishing gear and fishing vessel design, p. 25–29. Newfoundland and Labrador Institute of Fisheries and Marine Technology, St. John's, Newfoundland.

[6] Rogers, D. R., B. D. Rogers, J. A. de Silva, and V. L. Wright. 1994. Evaluation of shrimp trawls designed to reduce bycatch in inshore waters of Louisiana. School of Forestry, Wildlife, and Fisheries, Louisiana State Univ. Agricultural Center. Final report submitted to NMFS, St. Petersburg, FL. NOAA Award No. NA17FF0375-01, 230 p. Available from LSU library.

Table 3

Comparison of numbers and biomass of the most abundant species collected in bycatch reduction device (BRD) nets and corresponding control nets. SD is the standard deviation of the difference. Significance levels are 0.01 (**) and 0.05 (*). n=72. Superscripted letters denote significance levels of paired t–tests on log–transformed data: 0.01 (^{a}), 0.05 (^{b}).

	Numbers					Biomass (g)				
	Mean catch/tow		Percent catch			Mean catch/tow		Percent catch		
Species and BRD	Control	BRD	difference	SD	$P>t$–value	Control	BRD	difference	SD	$P>t$–value
Atlantic croaker										
Authement–Ledet	58.0	38.0	–34	67.9	0.01**^{a}	832.9	509.8	–39	711.1	0.01**
Lake Arthur	56.8	41.6	–27	63.4	0.05*^{b}	783.6	627.8	–20	450.9	0.01**^{a}
Cameron	58.5	29.6	–49	64.8	0.01**	859.8	570.2	–34	524.7	0.01**
Eymard	56.9	83.6	47	117.6	0.06^b	789.7	871.3	10	907.3	0.45
Spot										
Authement–Ledet	12.2	5.6	–54	12.4	0.01**^{a}	294.4	110.7	–62	318.0	0.01**^{a}
Lake Arthur	25.3	15.2	–40	52.3	0.10	575.5	372.7	–35	1,121.1	0.13
Cameron	12.0	9.8	–18	6.7	0.01**	322.5	269.4	–16	221.4	0.05*
Eymard	22.6	11.2	–50	49.4	0.05*^{a}	562.0	234.0	–58	1,137.9	0.02*^{a}
Sand seatrout										
Authement–Ledet	14.1	8.8	–38	26.3	0.09^b	101.0	63.5	–37	114.9	0.01**
Lake Arthur	9.8	8.5	–13	9.6	0.27	132.1	110.5	–16	138.8	0.19
Cameron	13.3	4.8	–64	14.5	0.01**^{a}	119.8	69.5	–42	105.5	0.01**^{a}
Eymard	17.0	20.1	19	21.7	0.22^b	165.5	160.1	–3	209.1	0.83
Hardhead catfish										
Authement–Ledet	22.3	11.0	–51	24.0	0.01**^{a}	625.5	244.9	–61	596.6	0.01**^{a}
Lake Arthur	26.2	20.8	–21	31.2	0.14^a	729.6	548.4	–25	380.4	0.01**^{a}
Cameron	35.3	14.4	–59	61.7	0.01**^{a}	960.0	549.1	–43	784.2	0.01**^{a}
Eymard	24.6	23.2	–6	51.3	0.82	802.0	393.7	–51	1,045.0	0.01**
Bay anchovy										
Authement–Ledet	29.3	18.3	–37	34.6	0.01**	40.1	22.1	–45	45.1	0.01**^{b}
Lake Arthur	23.3	14.7	–37	30.2	0.02*^{b}	35.3	25.0	–29	49.3	0.08^b
Cameron	27.8	7.0	–75	31.2	0.01**^{a}	36.7	9.5	–74	41.8	0.01**^{a}
Eymard	35.8	65.4	83	67.4	0.01**^{a}	43.4	80.8	86	79.8	0.01**^{a}
Gulf menhaden										
Authement–Ledet	12.8	10.0	–22	18.5	0.20	135.2	110.8	–18	170.0	0.23
Lake Arthur	7.8	8.5	9	14.2	0.67	96.0	106.7	11	161.7	0.57
Cameron	7.6	7.1	–7	12.5	0.71	82.1	82.0	0	126.3	0.99
Eymard	8.5	12.1	42	22.8	0.19	96.0	109.7	14	173.0	0.50
Blue crab										
Authement–Ledet	9.9	9.0	–9	6.9	0.25	430.3	352.1	–18	353.3	0.06
Lake Arthur	9.0	6.4	–28	7.6	0.01**	378.6	295.9	–22	326.0	0.03*
Cameron	11.4	9.3	–19	15.8	0.24	571.0	457.2	–20	888.7	0.28
Eymard	10.5	9.3	–12	6.3	0.09	574.4	410.0	–29	647.9	0.03*
Brown shrimp										
Authement–Ledet	60.0	46.7	–22	52.0	0.03*^{b}	332.4	270.7	–19	197.7	0.01**
Lake Arthur	64.3	48.2	–25	54.6	0.01**^{a}	333.8	268.8	–19	181.7	0.01**^{b}
Cameron	78.5	64.0	–19	72.4	0.09	375.6	315.6	–16	170.2	0.01**
Eymard	73.4	99.1	35	85.8	0.01**	356.3	438.6	23	273.4	0.01**
White shrimp										
Authement–Ledet	24.6	22.3	–9	22.9	0.40	150.3	146.3	–3	116.5	0.77
Lake Arthur	28.7	22.7	–21	20.9	0.02*^{a}	179.6	156.2	–13	137.4	0.15^a
Cameron	32.0	28.8	–10	24.6	0.28	202.9	184.5	–9	124.1	0.21
Eymard	25.4	37.0	46	47.5	0.04*^{b}	160.8	206.2	28	209.1	0.07

opening while the nets were being towed. Few fish were observed escaping from the Cameron and Lake Arthur BRD openings during these tests.

Although each BRD net contained escape openings, smaller species, such as the bay anchovy, could have escaped through the codend meshes. The devices may

have affected escape rates through the meshes by altering the shape of the codend. The percentage of fish and shrimp that escaped during trawling, as opposed to escaping during haulback, is also unknown. Watson et al. (1993) reported that most species escaped through escape openings during trawl haulback or when fish were crowded near the openings. Further diver evaluations are necessary to identify methods by which fish and shrimp escape.

Differences in catch rates of brown and white shrimp observed for many of the BRD's may have been due to species-specific behavior or size differences (or both). White shrimp swim more actively than brown shrimp during the day (Wickham and Minkler, 1975). The white shrimp caught by the control nets were larger, on average, than the brown shrimp, a finding that is typical for Louisiana catches (Keithly and Baron-Mounce[2]). In addition, the white shrimp caught in the spring were substantially larger than those in the fall.

These data are derived from a fishery-independent study; results from commercial shrimping could differ. Had the Eymard BRD been used in a larger trawl and without a hoop, the results might have been quite different. Many fish and shrimp may have been lost during haulback and although we had mechanical retrieval, a larger commercial vessel may have had faster retrieval. Although we trawled near shrimp boats whenever possible, at times no shrimp boats were present in a sampling area. When this was the case, we began trawling in an area where shrimp had been caught previously; if few or no shrimp were caught, we moved to another area. Moving short distances (one or two km) could result in very different catches. Because of time and fuel limitations, however, movements of very long distances were not feasible. Provided that shrimp were being caught, we did not relocate if large quantities of fishes or crabs were also present. In this situation, a shrimper would most likely relocate in an attempt to find more shrimp or cease shrimping until conditions in the area become more favorable. We found higher ratios of fish to shrimp when shrimp catches were low. Other studies have reported that bycatch ratios depend on shrimp abundance; when few shrimp are present, fishing times are longer and result in high catches of bycatch species (Adkins[7]). The 20-minute tows used in our study were three to six times shorter than those typically used in commercial operations. Longer tows would have necessitated decreasing the number of

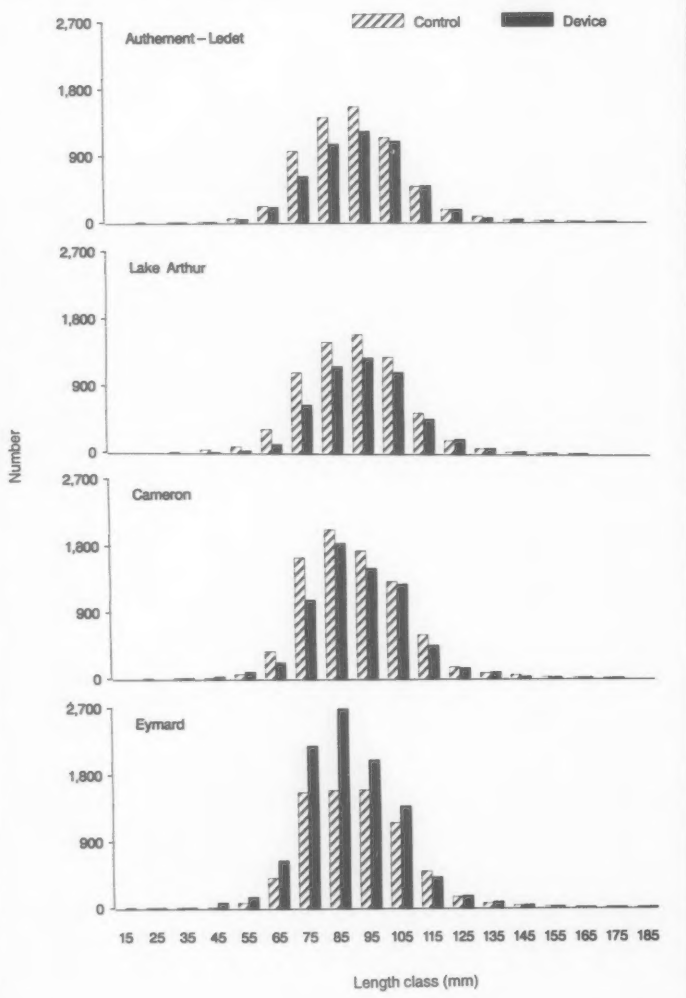

Figure 5

Length-frequency distribution of shrimp collected by the four devices and corresponding control nets.

[7] Adkins, G. 1989. A comprehensive assessment of bycatch in the Louisiana shrimp fishery. Final report to NMFS NA89WC-H-MF006. La. Dep. Wildlife Fish., Bourg, LA, 75 p.

Table 4

Comparisons of numbers and biomass of fish and shrimp collected by the four bycatch reduction device (BRD) nets and corresponding control nets in the three areas. SD is the standard deviation of the difference. Significance levels are 0.01 (**) and 0.05 (*). n=24. Superscripted letters denote significance levels of paired t-tests on log-transformed data: 0.01 (^{a}), 0.05 (^{b}).

BRD and type of catch	Area	Numbers: Mean catch/tow Control	Numbers: Mean catch/tow Device	Numbers: Percent catch difference	Numbers: SD	Numbers: $P>t$-value	Biomass (g): Mean catch/tow Control	Biomass (g): Mean catch/tow Device	Biomass (g): Percent catch difference	Biomass (g): SD	Biomass (g): $P>t$-value
Authement-Ledet											
Fish	L. Borgne	64.6	38.4	–41	45.7	0.01**^{a}	1,119.9	732.7	–35	588.1	0.01**^{a}
	L. Barre	202.7	134.8	–34	122.6	0.01**^{a}	2,425.2	1,458.9	–40	1,302.6	0.01**^{a}
	Calcasieu L.	244.0	153.8	–37	114.7	0.01**^{a}	4,080.2	2,200.8	–46	1,584.7	0.01**^{a}
Shrimp	L. Borgne	49.0	35.8	–27	21.3	0.01**	381.7	285.4	–25	178.1	0.01**
	L. Barre	90.5	86.7	–4	40.1	0.64	613.6	579.7	–6	233.9	0.49
	Calcasieu L.	115.1	85.5	–26	89.4	0.12^b	454.1	387.5	–15	300.6	0.29
Lake Arthur											
Fish	L. Borgne	65.4	47.0	–28	36.4	0.02*^{b}	1,365.2	1,072.9	–21	638.6	0.03*
	L. Barre	215.3	151.3	–30	149.3	0.05*	3,046.8	2,263.0	–26	2,441.9	0.13
	Calcasieu L.	233.4	205.6	–12	152.9	0.38	4,301.0	3,511.9	–18	1,235.3	0.01**^{a}
Shrimp	L. Borgne	57.0	47.0	–18	20.1	0.02*^{a}	389.1	350.6	–10	142.5	0.20^a
	L. Barre	95.3	70.3	–26	39.5	0.01**^{a}	649.4	509.4	–22	239.9	0.01**^{a}
	Calcasieu L.	127.4	95.4	–25	89.4	0.09	503.5	415.1	–18	252.1	0.10
Cameron											
Fish	L. Borgne	89.8	32.5	–64	113.0	0.02*^{a}	1,987.7	1,170.2	–41	1,361.9	0.01**^{a}
	L. Barre	188.9	98.6	–48	75.1	0.01**^{a}	2,693.3	1,776.4	–34	869.6	0.01**^{a}
	Calcasieu L.	266.5	133.9	–50	125.9	0.01**^{a}	4,524.6	3,257.7	–28	1,402.8	0.01**^{a}
Shrimp	L. Borgne	59.8	45.0	–25	23.6	0.01**^{a}	419.8	338.2	–19	145.7	0.01**^{b}
	L. Barre	108.2	97.5	–10	42.8	0.23	719.6	655.1	–9	225.8	0.18
	Calcasieu L.	164.8	136.6	–17	126.7	0.29^b	598.4	507.7	–15	278.4	0.12
Eymard											
Fish	L. Borgne	81.6	114.4	40	83.9	0.07^a	1,970.0	1,288.0	–35	1,394.5	0.03*
	L. Barre	201.8	301.0	49	193.1	0.02*^{a}	2,724.3	2,554.9	–6	2,158.8	0.70
	Calcasieu L.	287.8	303.7	6	195.7	0.69	4,246.5	3,376.1	–20	2,493.5	0.10^b
Shrimp	L. Borgne	52.7	84.1	60	79.2	0.06^a	365.2	510.4	40	343.3	0.05*
	L. Barre	97.5	120.6	24	34.1	0.01**^{a}	672.8	730.8	9	166.2	0.10^b
	Calcasieu L.	146.5	204.5	40	145.4	0.06^a	513.5	694.8	35	450.7	0.06^a

trips or evaluating fewer BRD's. Increasing the trawl-tow duration decreases the ability of a fish to maintain swimming speed (Bainbridge, 1960). Reductions over longer tow periods may differ; if most reduction occurs during haulback, fish may be too exhausted to escape. Longer tows also increase the chances of catching large quantities of fish and shrimp that may clog the net and cause organisms to be released from the BRD.

In terms of abundances and size distributions, bycatch varied between the areas and seasons; some species were very abundant in one or two areas. The capability of a BRD to reduce fish or shrimp depends on the species assemblage present in an area. A BRD may work well in one area under certain conditions but perform poorly in another area owing to assemblage differences. Species-specific size selectivity has been reported in other studies (e.g. Rulifson et al., 1992). Of the four BRD's, the Cameron had the best overall fish reduction. However, if spot and gulf menhaden were the most abundant species in an area, the Authement-Ledet may be a better choice. Bycatch reduction devices may have to be selected for particular areas or seasons, depending on the type and size distributions of predominant bycatch species, because a particular device may not be as effective in all areas or at all times of the year.

The high shrimp losses from the BRD's evaluated in this study would most likely be unacceptable for commercial operations. However, further modifications to these devices, such as altering the size or location of escape openings, could reduce these losses.

Table 5

Comparison of numbers and biomass of fish and shrimp collected by the four bycatch reduction device (BRD) nets and corresponding control nets for the different seasons. S_D is the standard deviation of the difference. Significance levels are 0.01 (**) and 0.05 (*). n=36. Superscripted letters denote significance levels of paired t-tests on log-transformed data: 0.01 (^{a}), 0.05 (^{b}).

BRD and type of catch	Season	Numbers: Mean catch/tow Control	Numbers: Mean catch/tow Device	Numbers: Percent catch difference	Numbers: SD	Numbers: $P>t$-value	Biomass (g): Mean catch/tow Control	Biomass (g): Mean catch/tow Device	Biomass (g): Percent catch difference	Biomass (g): SD	Biomass (g): $P>t$-value
Authement-Ledet											
Fish	spring	218.3	137.8	–37	127.7	0.01**^{a}	2,895.3	1,667.1	–42	1,374.2	0.01**^{a}
	fall	122.6	80.2	–35	65.5	0.01**^{a}	2,188.3	1,261.2	–42	1,354.2	0.01**^{a}
Shrimp	spring	116.6	92.1	–21	73.2	0.05*^{b}	650.3	556.6	–14	288.7	0.06^b
	fall	53.1	46.5	–12	36.4	0.29^b	316.0	278.5	–12	180.4	0.22
Lake Arthur											
Fish	spring	216.1	165.2	–24	157.1	0.06	3,459.1	2,761.7	–20	2,119.1	0.06^b
	fall	126.7	104.2	–18	80.9	0.10^b	2,349.6	1,803.6	–23	893.6	0.01**^{b}
Shrimp	spring	124.8	96.5	–23	75.4	0.03*^{b}	642.3	541.1	–16	244.8	0.02*^{b}
	fall	61.7	45.3	–27	31.0	0.01**^{a}	385.7	308.9	–20	190.3	0.02*^{a}
Cameron											
Fish	spring	203.1	106.5	–48	101.5	0.01**^{a}	3,296.6	2,229.1	–32	1,187.8	0.01**^{a}
	fall	160.3	70.2	–56	118.9	0.01**^{a}	2,840.4	1,907.1	–33	1,290.5	0.01**^{a}
Shrimp	spring	157.9	134.9	–15	104.4	0.19^a	773.1	677.9	–12	252.8	0.03*^{a}
	fall	64.0	51.2	–20	35.8	0.04*	385.4	322.8	–16	184.8	0.05*
Eymard											
Fish	spring	233.8	301.1	29	197.3	0.05*^{a}	3,386.9	2,773.0	–18	2,029.4	0.08^b
	fall	147.1	178.3	21	131.7	0.16^b	2,573.8	2,039.7	–21	2,117.8	0.14
Shrimp	spring	145.1	199.8	38	119.5	0.01**^{a}	716.1	883.0	23	380.7	0.01**^{a}
	fall	52.6	73.0	39	65.9	0.07^a	318.2	407.7	28	294.3	0.08

The BRD nets tested here did not appear to slow water flow in the trawl net. Other studies, however, have indicated that flow rate around and through the BRD may be a key factor in fish and shrimp escapement. Watson et al. (1993) found that juvenile fish could exit a BRD at flow rates between 0.2 and 0.5 m/sec. However, shrimp accumulated in areas of reduced flow and crawled along the webbing against the flow to escape some devices (Watson et al., 1993). Devices can be designed to create a 0.2 to 0.5 m/sec flow rate, but debris can alter the flow rate and affect BRD performance. The ability to sustain swimming appears to be related to length, but this relationship often differs for each species (Bainbridge 1960). Further testing is necessary to acquire escape flow rates for the major species of concern.

Reduction rates for numbers and biomass of many species differed for the four BRD's, reflecting size-dependent selectivity. Escape rates of different species also varied considerably owing to differences in size and behavior. These differences, coupled with the high variability in organisms between areas, indicate that the performance of BRD's should be evaluated at the species and size level.

Future studies should continue to involve members of the industry. The advisory committee provided suggestions and valuable insight that greatly enhanced the success of this project and the acceptability of the results. Other studies have reported successful industry involvement (Rulifson et al., 1992; McKenna and Monaghan[4]). The design and construction of BRD's should be a dynamic process which will benefit from the cooperation of industry, research, and management personnel.

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Global population structure of yellowfin tuna, *Thunnus albacares*, inferred from allozyme and mitochondrial DNA variation

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Abstract.—Yellowfin tuna, *Thunnus albacares*, were sampled from one region of the Atlantic Ocean, two regions of the Indian Ocean, and six regions of the Pacific Ocean. One of the Indian Ocean collections could not be allozymically analyzed; the remaining eight collections were examined for four polymorphic allozyme loci (*ADA**, *FH**, *GPI-A**, and *GPI-B**, n=540 to 677). All nine collections were examined for mitochondrial DNA variation (n=767), with two restriction enzymes (*Bcl* I and *Eco* RI) that detect polymorphic restriction sites in yellowfin tuna. Allele frequencies at three of the allozyme loci were homogeneous across collections, whereas *GPI-A** showed highly significant differentiation (P<0.001). The *GPI-A** data, taken together with the geographic location of the collections, suggested the existence of at least four yellowfin tuna stocks: Atlantic Ocean, Indian Ocean, west-central Pacific Ocean, and east Pacific Ocean. Mitochondrial DNA differentiation was more limited, but spatial heterogeneity of the 24 observed haplotypes over the nine regions (P=0.048) and three oceans (P=0.009) was significant. The mtDNA data did not differentiate west-central Pacific Ocean collections from east Pacific Ocean collections but did support the separation of Atlantic Ocean, Indian Ocean, and Pacific Ocean stocks.

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The yellowfin tuna, *Thunnus albacares* (Bonnaterre), supports important fisheries in tropical and subtropical oceans. Catches have increased from about 600,000 metric tons (t) in 1982 and 1983 to about 1,100,000 t in 1993 and 1994; in 1994, about 63% of the catch came from the Pacific Ocean, about 24% from the Indian Ocean, and 14% from the Atlantic Ocean (FAO, 1996). Given the size and circumglobal nature of the resource, there is considerable management interest in determining stock structures.

It is only comparatively recently that yellowfin tuna has been recognized as a single species (Gibbs and Collette, 1967); its high degree of morphological variation led Jordan and Evermann (1926) to recognize seven yellowfin tuna species. However, a major morphometric study by Royce (1964) revealed that intra-oceanic differences could be greater than interoceanic differences and that several characters showed clinal variation. He concluded that the morphometric data are best explained by a single worldwide pantropical species, a conclusion confirmed by Gibbs and Collette (1967).

Most stock structure studies of yellowfin tuna have focused on the large Pacific Ocean component of the catch. Here, tagging experiments indicated that yellowfin tuna usually migrate hundreds rather than thousands of kilometers and that their movements do not range far both east-west or north-south (Joseph et al., 1964; Bayliff, 1979; Hunter et al., 1986; Lewis, 1992). Morphometric studies have provided commensurate results, with Mexico and Ecuador fish being much more similar to one another than to fish from the central (Hawaii) and western (Australia, Japan) Pacific (Schaefer, 1991). Studies of the microchemical composition of larval portions of otoliths in West Pacific fish (Indonesia, Philippines, Coral Sea, Hawaii) have shown some differences, indicating that such analyses may be useful in determination of spawning origins (Gunn and Ward[1]; Gunn[2]). Genetic studies of four to five polymorphic

[1] Gunn, J. S., and R. D. Ward. 1994. The discrimination of yellowfin tuna sub-populations within the AFZ. Phase 1: a pilot study to determine the extent of genetic and otolith microchemical variability in populations from different parts of the Pacific and Indian Oceans. Final Report (91/27) to Fisheries Research and Development Corporation, Deakin, ACT, Australia.

[2] Gunn, J. S. 1996. CSIRO Division of Marine Research, Hobart, Tasmania, Australia. Unpubl. data.

allozyme loci in Pacific Ocean collections have shown significant spatial heterogeneity at one locus (*GPI-A**); the common allele in western and central regions differed from that in the east (Sharp, 1978; Ward et al., 1994). This finding either indicates the existence of two reproductively isolated groups of yellowfin tuna in the Pacific Ocean or suggests that selection pressures are different in the two regions. There is no evidence of mitochondrial DNA (mtDNA) differentiation between eastern Pacific and western Pacific yellowfin tuna (Scoles and Graves, 1993; Ward et al., 1994).

In the Atlantic Ocean, where it was once assumed that there were separate eastern and western stocks, recent taggings of large yellowfin tuna have resulted in 15 trans-Atlantic recoveries (ICCAT, 1992b); a single stock is now assumed (ICCAT, 1995). There have been no genetic comparisons of eastern and western Atlantic yellowfin tuna.

The extent of genetic differentiation of yellowfin tuna from different oceans has been little studied. Suzuki (1962) found no differences in the incidence of the Tg2 blood group antigen in fish from the equatorial Pacific and Indian Oceans. Scoles and Graves (1993) found no significant differentiation in mtDNA from one west Atlantic collection and five Pacific collections (each of 20 fish). Here we compare genetic variation in collections from the Pacific, Indian, and Atlantic oceans. We used larger sample sizes than those used in the study by Scoles and Graves (1993) and examined both allozyme and mtDNA variation to see if the increased statistical power would enable us to reject the null hypothesis of no interoceanic genetic differentiation.

Materials and methods

Samples were collected from one region of the Atlantic Ocean, two regions of the Indian Ocean, and six regions of the Pacific Ocean. Details of most of the Pacific collections (Philippines, Coral Sea, Kiribati, Hawaii, California, and Mexico) are given in Ward et al. (1994). For the present paper, the 1991 and 1992 Hawaii collections were pooled. A second Philippines collection was collected in October–December 1994. This showed no significant genetic differentiation from the earlier collection; therefore the two collections were pooled for our study. The Atlantic collection was taken from the Caribbean Sea (Gulf of Mexico, approx. 28°N, 88°W) in September 1993. The Indian Ocean collections were taken from off Sri Lanka (approx. 6°N, 80°E) and off the Seychelles (approx. 7°S, 52°E) in December 1994. White muscle samples were flown (airfreight) frozen to Hobart and stored at –80°C.

Specimens were studied by allozyme and mtDNA analysis. The experimental methods are given in Ward et al. (1994). Four allozyme loci known to be polymorphic in white muscle were examined: *ADA** (adenosine deaminase, EC 3.5.4.4), *FH** (fumarate hydratase, EC 4.2.1.2), *GPI-A**, and *GPI-B** (glucose-6-phosphate isomerase, EC 5.3.1.9). MtDNA variation was examined by using two restriction enzymes (*Bcl* I and *Eco* RI) known to discriminate most of the mtDNA haplotypes revealed by eight restriction enzymes in an earlier survey (*Bam* HI, *Ban* I, *Bcl* I, *Eco* RI, *Hind* III, *Pvu* II, *Sal* I, and *Xho* I—see Ward et al., 1994).

The homogeneity of allele and haplotype frequencies of the collections was tested by the randomized Monte Carlo chi-square procedure of Roff and Bentzen (1989). For each test, 2,000 randomizations of the data were carried out, each giving a randomized chi-square value (χ^2_{null}). The probability that the null hypothesis of genetic homogeneity was correct was given by $P = n/2{,}000$, where n is the number of randomizations that generate $\chi^2_{null} \geq \chi^2$ and where χ^2 is the chi-square value given by the actual observations.

The extent of genetic differentiation among collections was quantified by Nei's gene diversity statistic G_{ST} (Nei, 1987), which estimates the proportion of total genetic variation attributable to differentiation between populations. For each allozyme locus, G_{ST} was estimated as $(H_T - H_S)/H_T$, where H_T represents total heterozygosity and H_S is average (Hardy-Weinberg expected) population heterozygosity. The mtDNA data were analyzed in a similar way, treating haplotypes as alleles and H_T and H_S as diversity estimates. The proportion or magnitude of G_{ST} generated by sampling error, which we have termed $G_{ST.null}$, was estimated with a bootstrapping program, with the observed allele or haplotype frequencies and collection sizes. Simulations were run 1,000 times to provide a mean value of $G_{ST.null}$ and a standard deviation. The probability of obtaining a value of $G_{ST.null}$ as large or larger than that obtained from the actual observations of G_{ST} was given by $P = n/1{,}000$, where n is the number of randomizations that generate $G_{ST.null} \geq G_{ST}$. Values of P less than 0.05 indicated significant differentiation between areas that could not be explained by sampling error alone.

Bonferroni adjustments of significance levels, to correct for multiple tests, were carried out with the sequential procedure advocated by Hochberg (1988). Tests are ordered according to their probability value. The highest probability value, P_m, is compared with the significance value α. Here we initially set $\alpha = 0.05$. If $P_m \geq \alpha$, that test is judged to be nonsignificant, and comparisons continue with subsequent probabilities, each compared with a modified signifi-

cance level = $\alpha/(1+i)$, where i is the number of tests already performed. When a test is significant, it and all subsequent tests are deemed significant.

Cluster analysis of the allozyme allele frequency data and the mtDNA haplotype frequency data used the UPGMA (unweighted pair-group method using averages) algorithm with Nei's (1978) unbiased genetic distance measure, as implemented in BIOSYS-1 (Swofford and Selander, 1981).

Estimates of mtDNA nucleotide sequence diversity and divergence (Nei and Tajima, 1981; Nei, 1987) were made with REAP vers.4.0 (see McElroy et al., 1992), and population divergences were clustered by using UPGMA.

Results

The Seychelles muscle samples were partially degraded on arrival, and could not be confidently screened for allozyme determinations, although mtDNA analysis presented no problems. Because it is sometimes difficult to distinguish tuna species, we usually find a small percentage (3–5%) of non-yellowfin tunas among nominal yellowfin tuna collections. These misidentified fish can be recognized by aberrant allozyme (Graves et al., 1988; Elliott and Ward, 1995) and mtDNA patterns (Grewe[3]). For example, five (3.7%) of the 135 Philippines samples collected in 1994 proved to be bigeye tuna, *Thunnus obesus*. However, at times, the proportion of misidentified fish can be much higher: 18 (46.2%) of the 39 "yellowfin tuna" from Sri Lanka proved to be bigeye tuna. The misidentified fish were excluded from the following analyses.

Allozyme allele frequencies at four polymorphic loci (*ADA**, *FH**, *GPI-A**, *GPI-B**) for eight collections (Table 1) and mtDNA haplotype frequencies for nine collections (Table 2) were determined.

No significant deviations from Hardy-Weinberg expectations were recorded for any allozyme locus. Heterogeneity chi-square analyses (Table 3) of allele frequencies revealed no significant differentiation for three loci (*ADA**, *FH**, and *GPI-B**), but highly significant heterogeneity at the fourth locus, *GPI-A** ($P<0.001$, $\alpha=0.0125$). Genetic diversity (G_{ST}) analyses (Table 3) indicated that for *ADA**, *FH**, and *GPI-B**,

[3] Grewe, P. M. 1993. CSIRO Division of Marine Research, Hobart, Tasmania, Australia. Unpubl. data.

Table 1

Allozyme allele frequencies and sample sizes (n). GOM = Gulf of Mexico, S.Lan. = Sri Lanka, Philipp. = Philippines, Cl. Sea = Coral Sea, Calif. = California.

		Atlantic	Indian	Pacific					
Locus	Allele	GOM	S. Lan.	Philipp.	Cl. Sea	Kiribati	Hawaii	Calif.	Mexico
*ADA**	125	0.005	—	0.003	—	—	—	—	—
	115	0.414	0.310	0.330	0.306	0.399	0.361	0.317	0.359
	100	0.548	0.643	0.622	0.638	0.567	0.609	0.671	0.628
	85	0.033	0.048	0.045	0.056	0.034	0.030	0.012	0.013
	n	105	21	176	98	89	115	41	39
*FH**	130	0.118	0.091	0.081	0.117	0.086	0.076	0.051	0.075
	100	0.875	0.909	0.910	0.878	0.900	0.920	0.949	0.925
	75	0.007	—	0.009	0.005	0.014	0.004	—	—
	n	68	11	111	98	70	112	39	29
*GPI-B**	–20	0.015	—	—	—	—	0.004	—	—
	–60	0.180	0.167	0.176	0.163	0.233	0.113	0.187	0.231
	–100	0.806	0.833	0.824	0.837	0.767	0.878	0.813	0.769
	–125	—	—	—	—	—	0.004	—	—
	n	103	21	176	98	88	115	40	39
*GPI-A**	145	—	—	0.003	—	—	—	—	—
	135	0.045	—	0.015	0.036	0.011	0.035	0.122	0.077
	100	0.624	0.286	0.651	0.683	0.673	0.609	0.305	0.231
	75	0.332	0.714	0.328	0.281	0.316	0.357	0.573	0.692
	40	—	—	0.003	—	—	—	—	—
	n	101	21	175	98	87	115	41	39

slightly less than 1% of the observed diversity arose from differences between collections and could be attributed to sampling error alone ($G_{ST.null}$). For *GPI-A**, the observed value of G_{ST}, at 12%, was much larger than the value attributable to sampling error (about 1%). The "true" G_{ST} estimate of *GPI-A** —the difference between G_{ST} and $G_{ST.null}$ —is thus around 11%, indicating that about 11% of the observed diversity at the *GPI-A** locus comes from differences between collections.

The *GPI-A** heterogeneity (Fig. 1) was further explored by comparing all collections pairwise (Fig. 2; Table 4). This comparison essentially revealed two groups of collections: 1) the west-central Pacific Ocean and the Atlantic Ocean (Gulf of Mexico) collections; and 2) the Indian Ocean (Sri Lankan) and eastern Pacific Ocean (Californian and Mexican) collections. Within each of these two groups there was no significant differentiation, but between them differentiation was marked. This conclusion holds after Bonferroni corrections of α levels for multiple tests. The *GPI-A**100 allele was the most frequent allele in the west-central Pacific Ocean and the Atlantic Ocean group, whereas the *GPI-A**75 allele was the more frequent allele in the Indian Ocean and the eastern Pacific Ocean group. The genetic differentiation of the Atlantic Ocean collection from the Indian Ocean collection suggests that fish from these areas constitute separate stocks; the separation of the Indian Ocean collection from the west-central Pacific Ocean collections suggests that fish from these areas constitute separate stocks; the separation of the west-central Pacific Ocean collections from the eastern Pacific Ocean collections suggests that fish from these areas constitute separate stocks; and the separation of the eastern Pacific Ocean collections

Table 2

Mitochondrial DNA haplotype frequencies (*Bcl* I and *Eco* RI haplotypes respectively), sample sizes (n), haplotype diversities (h) and percent nucleotide diversities (% n.d.). Abbreviations are defined in Table 1. Seych. = Seychelles.

		Atlantic	Indian		Pacific					
Locus	Haplotype	GOM	Seych.	S. Lan.	Philipp.	Cl. Sea	Kiribati	Hawaii	Calif.	Mexico
mtDNA	AA	0.266	0.319	0.381	0.286	0.340	0.443	0.276	0.294	0.325
	AB	0.543	0.407	0.333	0.509	0.402	0.364	0.537	0.463	0.425
	AC	—	—	—	—	—	0.011	0.015	—	—
	AF	—	0.011	—	—	—	—	0.007	0.049	0.025
	AG	—	—	—	0.006	—	—	0.007	—	—
	BA	0.011	0.011	—	0.025	0.010	—	0.007	0.049	—
	BB	0.064	0.066	—	0.031	0.052	0.068	0.060	0.073	0.025
	CA	0.032	0.011	—	0.031	0.062	0.011	0.015	0.024	0.050
	CB	0.021	0.088	0.286	0.068	0.103	0.057	0.045	0.024	0.125
	CO	0.021	—	—	—	—	—	—	—	—
	DB	—	—	—	—	0.010	—	—	—	—
	EB	—	—	—	—	0.021	—	—	—	—
	LB	—	—	—	—	—	0.011	—	—	—
	MB	—	—	—	—	—	0.011	—	—	—
	NB	—	—	—	0.019	—	—	0.007	—	—
	PB	—	—	—	—	—	—	0.015	0.024	—
	OA	—	—	—	0.006	—	—	—	—	0.025
	OB	—	—	—	0.006	—	0.023	—	—	—
	QA	—	0.011	—	—	—	—	—	—	—
	QB	0.011	0.011	—	—	—	—	0.007	—	—
	WA	0.032	0.033	—	—	—	—	—	—	—
	ZB	—	0.022	—	0.006	—	—	—	—	—
	A2B	—	—	—	0.006	—	—	—	—	—
	Q2B	—	0.011	—	—	—	—	—	—	—
	n	94	91	21	161	97	88	134	41	40
	h	0.634	0.727	0.695	0.655	0.712	0.670	0.633	0.705	0.712
	% n.d.	0.998	1.263	1.017	1.017	1.174	1.027	0.901	1.099	1.047

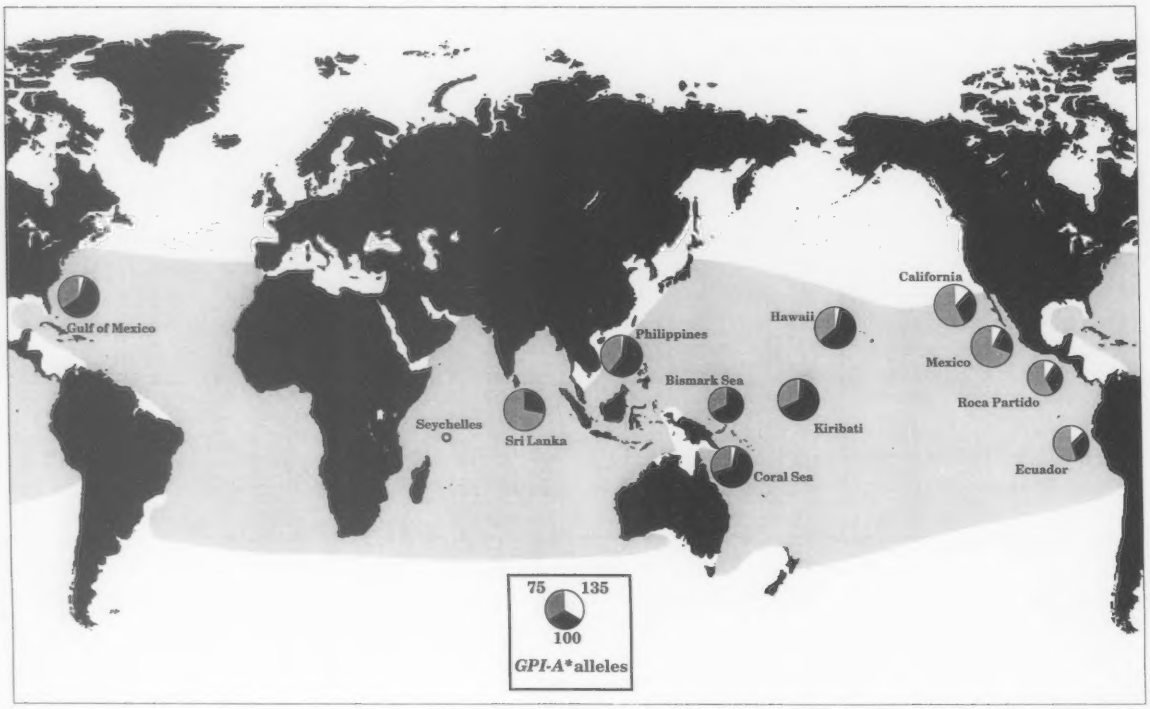

Figure 1

Map of sample sites showing *GPI-A** gene frequencies in yellowfin tuna, *Thunnus albacares*. Larger circles represent our data (Table 1), the three smaller circles (Bismark Sea, Roca Partido, and Ecuador) data are from Sharp (1978). The location of the Seychelles sample, examined for mtDNA variation but not for *GPI-A** variation, is identified. The shaded area represents the approximate global distribution of yellowfin tuna.

from the Atlantic Ocean collection suggests that these fish constitute separate stocks. Thus the *GPI-A** data, taken together with the spatial orientation of these collections, indicate the existence of at least four yellowfin tuna stocks: Atlantic Ocean, Indian Ocean, west-central Pacific Ocean, and east Pacific Ocean.

Six of the 24 mtDNA haplotypes (CO, QA, WA, ZB, A2B, Q2B, see Table 2) were not recorded in the earlier survey of Ward et al. (1994) but were rare (frequencies less than 3.5%). Fragment sizes for most haplotypes are given in Ward et al. (1994), but a full list is available on request. Haplotype (nucleon) diversities per collection ranged from 0.633 to 0.727 (mean estimate of 0.683) (Table 2). Percent nucleotide diversities per collection ranged from 0.998 to 1.263 (mean estimate of 1.061) (Table 2).

Table 3

Analyses of genetic differentiation among the samples.

Locus	Number of fish	Number of alleles/haplotypes	Heterogeneity χ^2 analysis		Genetic diversity analysis		
			χ^2	P	G_{ST}	$G_{ST.null}$±SD	P
*ADA**	684	4	17.326	0.666	0.006	0.008 ±0.004	0.569
*FH**	538	3	9.241	0.821	0.006	0.011 ±0.008	0.736
*GPI-B**	680	4	29.256	0.128	0.008	0.008 ±0.005	0.370
*GPI-A**	677	5	131.416	<0.001	0.118	0.008 ±0.004	<0.001
mtDNA	767	24	227.743	0.048	0.023	0.015 ±0.005	0.071

Table 4

Pairwise comparisons of *GPI-A** allele frequencies (*P* above, chi square below). GOM = Gulf of Mexico.

	GOM	Sri Lanka	Philippines	Coral Sea	Kiribati	Hawaii	California	Mexico
GOM	—	<0.001	0.156	0.468	0.129	0.769	<0.001	<0.001
Sri Lanka	21.700	—	0.001	<0.001	<0.001	0.001	0.031	0.214
Philippines	5.938	24.115	—	0.264	0.965	0.291	<0.001	<0.001
Coral Sea	1.586	28.644	4.870	—	0.270	0.237	<0.001	<0.001
Kiribati	3.905	22.586	1.196	2.630	—	0.203	<0.001	<0.001
Hawaii	0.493	19.130	4.635	2.824	3.280	—	<0.001	<0.001
California	24.850	6.047	46.871	35.021	37.324	25.374	—	0.289
Mexico	34.934	3.579	51.193	46.401	44.495	33.366	2.526	—

Table 5

Pairwise comparisons of mtDNA haplotype frequencies (*P* above, chi square below). GOM = Gulf of Mexico.

	GOM	Seychelles	Sri Lanka	Philippines	Coral Sea	Kiribati	Hawaii	California	Mexico
GOM	—	0.277	0.009	0.089	0.025	0.009	0.384	0.285	0.076
Seychelles	14.079	—	0.549	0.062	0.188	0.212	0.200	0.478	0.707
Sri Lanka	23.110	9.697	—	0.269	0.369	0.171	0.111	0.025	0.612
Philippines	19.823	22.529	14.013	—	0.223	0.025	0.428	0.207	0.597
Coral Sea	18.085	16.020	7.785	15.432	—	0.087	0.053	0.092	0.683
Kiribati	21.523	17.480	11.862	22.368	15.117	—	0.086	0.093	0.253
Hawaii	13.801	18.574	18.760	15.533	19.300	18.754	—	0.667	0.378
California	11.888	11.682	13.905	17.165	14.055	16.316	8.888	—	0.383
Mexico	15.622	9.718	5.017	10.188	7.203	12.214	13.080	8.473	—

A chi-square test (Table 3) showed that the mtDNA haplotype variation across all nine regions was just significant (α=0.05, P=0.048, with the standard 2,000 replicates, and P=0.045, with 10,000 replicates). Genetic diversity analysis (Table 3) gave a result bordering on significance (P=0.071, with a "true" G_{ST} of about 1%). All collections were compared pairwise with chi-square tests (Table 5) to determine which collections contributed most to the marginal chi-square differentiation. Although some pairs appeared significantly different (e.g. Gulf of Mexico versus Sri Lanka, P=0.009; Gulf of Mexico versus Kiribati, P=0.009), none was significant after Bonferroni adjustments for table-wide comparisons.

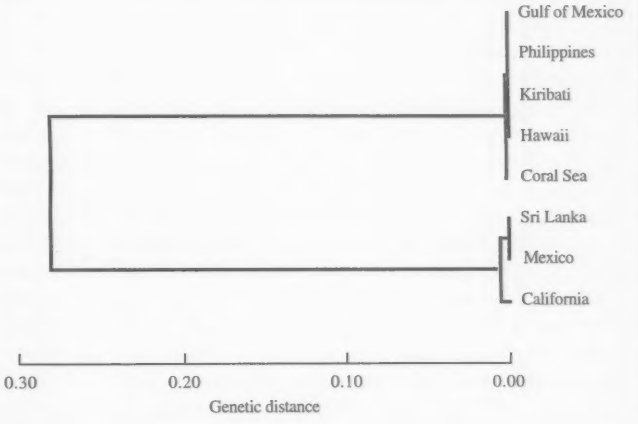

Figure 2

UPGMA phenogram for the *GPI-A** locus constructed from Nei's (1978) unbiased genetic distance.

Two UPGMA dendrograms were estimated. One, based on mtDNA haplotype frequencies alone (Fig. 3A), showed the maximal genetic-distance estimates among collections to be about 0.05 — much less than the major *GPI-A** split of nearly 0.30 (Fig. 2). The second, based on percent sequence divergence (Fig. 3B), confirmed the high degree of similarity among the collections. After correcting for within-collection nucle-

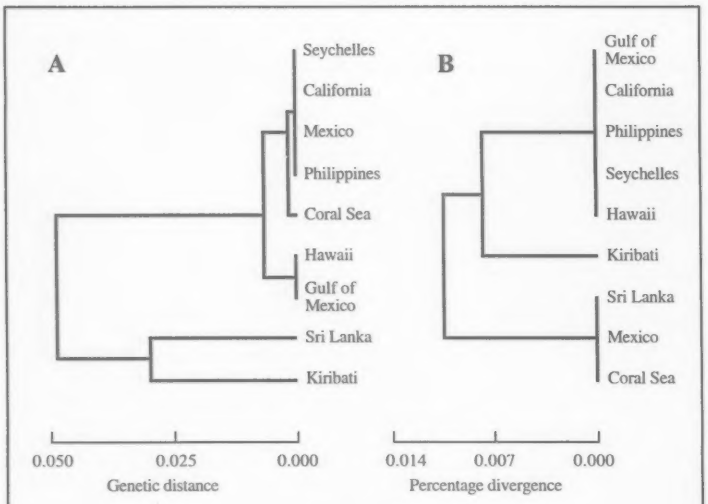

Figure 3

UPGMA phenograms for the mtDNA data with (**A**) unbiased genetic distance (Nei 1978), and (**B**) percentage nucleotide divergence (Nei, 1987).

otide divergence, pairwise nucleotide divergence ranged from 0.040% to –0.025% (mean 0.004%). There was little correspondence between these two mtDNA dendrograms, and this lack of correspondence, together with the low distances observed, suggests that the tree topologies are unreliable.

Because there is no significant mtDNA differentiation between the six Pacific Ocean collections (Table 5; and Ward et al., 1994) nor between the two Indian Ocean collections (Table 5), the collections within each ocean were pooled to test for interoceanic differences. A comparison of the three oceans yielded a chi-square analysis that was significant (P=0.009, α=0.05) and a genetic diversity analysis bordering on significance (observed G_{ST}=0.010, $G_{ST.null}$=0.005 ±0.003, P=0.059). A pairwise comparison of the oceans showed that all pairs were significant (Indian versus Atlantic, P=0.047, α=0.05; Pacific versus Atlantic, P=0.017; Pacific versus Indian, P=0.009).

Finally, the mtDNA data were analyzed to see whether they offered any support to the conclusion from the *GPI-A** data that there are (at least) four yellowfin tuna stocks. The four putative stocks consisted of the following units: Atlantic (Gulf of Mexico), Indian (Seychelles and Sri Lanka), west-central Pacific (Coral Sea, Kiribati, Philippines, Hawaii), and east Pacific (California and Mexico). Chi-square analysis of mtDNA data from these four regions indicated limited but significant (P=0.024) heterogeneity. Although none of the six pairwise comparisons was significant following Bonferroni correction to α levels (west-central Pacific versus east Pacific, P=0.456, α=0.05; Indian versus east Pacific, P=0.316, α=0.025; Atlantic versus east Pacific, P=0.119, α=0.017; Atlantic versus Indian, P=0.047, α=0.0125; Atlantic versus west-central Pacific, P=0.032, α=0.010; Indian versus west-central Pacific, P=0.0135, α=0.008), the three pairwise comparisons of the Atlantic Ocean, Indian Ocean, and west-central Pacific all showed P-values less than 0.05.

Clearly, the mtDNA data do not differentiate west-central Pacific Ocean collections from east Pacific Ocean collections but, considering the interocean analyses alone, do provide some support for the delineation of Atlantic Ocean, Indian Ocean, and Pacific Ocean stocks.

Discussion

Samples of yellowfin tuna from the Pacific, Indian, and Atlantic oceans were compared with respect to four polymorphic allozyme loci and with respect to mtDNA variants.

No significant allele frequency differences were observed for three of the allozyme loci, but the fourth locus, *GPI-A**, showed considerable differentiation. Across all collections, the "true" G_{ST} indicated that about 11% of the variation at this locus was attributable to differences between collections. Two genetically distinguishable groups were apparent. One consists of eastern Pacific Ocean and Indian Ocean fish, with a high frequency of the *GPI-A**75 allele, the other of Atlantic Ocean and west-central Pacific Ocean fish, with a high frequency of the *GPI-A**100 allele. Because there are no migration routes between the eastern Pacific Ocean (California and Mexico) and the Indian Ocean that avoid the west-central Pacific Ocean, and between the Atlantic Ocean and west-central Pacific Ocean that avoid the Indian Ocean, there is reason to believe that there are at least four stocks of yellowfin tuna: Atlantic Ocean, Indian Ocean, west-central Pacific Ocean, and eastern Pacific Ocean.

Sharp (1978) also examined *GPI-A** allele frequencies in western and eastern Pacific populations. His *GPI-A** allele frequencies for collections from Ecuador and Mexico were very similar to our California and Mexico frequencies, and his *GPI-A** frequencies

from the Bismarck Sea in the western Pacific were very similar to our western Pacific Ocean frequencies (Ward et al., 1994), supporting the separation of western and eastern Pacific stocks. Another allozyme study (Fujino, 1970) failed to find differences between Hawaiian and eastern Pacific fish for an esterase and for transferrin, although the esterase was nearly monomorphic.

We interpret the *GPI-A** differentiation as being indicative of stock differences, resulting from restricted gene exchange between the four identified regions. However, the alternative explanation, that of differential selection in the presence of gene flow, cannot be ruled out. Indeed, the very limited mtDNA differentiation observed could be held to support this interpretation. Microsatellite analysis, currently underway, may help to resolve this question. Selection acting on these noncoding genetic markers is presumed to be minimal or nonexistent; therefore microsatellite differentiation paralleling the *GPI-A** differentiation would suggest drift of neutral *GPI-A** alleles, whereas lack of microsatellite differentiation would indicate significant gene flow and thereby implicate selection as the cause of the *GPI-A** differentiation. Pogson et al. (1995) have recently suggested that the highly heterogeneous distribution of anonymous nuclear RFLP markers among populations of cod, *Gadus morhua*, reflects limited gene flow and that the much more homogeneous distribution of allozyme alleles reflects stabilizing selection rather than extensive gene flow. Such an argument applied to yellowfin tuna data would interpret the *GPI-A** heterogeneity as indicative of limited gene flow, and the *ADA**, *FH**, and *GPI-B** homogeneity as indicative of stabilizing selection at these three loci.

Differences between collections in mtDNA was only just significant (P=0.048), with a "true" G_{ST} value across all nine collections of around 1%. When collections were pooled within oceans, i.e. the three groups (Atlantic, Indian, and Pacific), significant differentiation was detected (P=0.009), although the "true" G_{ST} was only of the order of 0.5%. All three pairwise ocean comparisons were statistically significant. However, because collections within oceans did not always pool together in the distance dendrograms (Fig. 3), possibly because of limited sample sizes, it would clearly be useful to have more data to confirm (or refute) this evidence of interoceanic differentiation. When collections were pooled into the four putative stocks indicated by the *GPI-A** data, limited but significant heterogeneity in mtDNA haplotype frequencies was apparent (P=0.024), but there were no significant pairwise comparisons.

Scoles and Graves (1993) were unable to detect significant mtDNA differentiation between Pacific and Atlantic yellowfin tuna, whereas the probability of homogeneity in our tests of these two oceans was only 0.017. However, they adopted a different test strategy. Instead of examining relatively large numbers of fish (our study: Pacific fish, n=561; Atlantic fish, n=94) with relatively few restriction enzymes (n=2, but known to detect polymorphic sites), they chose to examine relatively few fish (Pacific fish, n=100; Atlantic fish, n=20) with a relatively large number of restriction enzymes (n=12, which included the two enzymes we used). Given that the common 12-enzyme haplotype in Scoles and Graves' study comprised 52 fragments or 304 bp and that the common 2-enzyme haplotype in our study comprised 7 fragments or 42 bp (see Ward et al., 1994) and that the mean size of the yellowfin tuna mtDNA genome is about 16,702 bp (Scoles and Graves [1993] estimate=16,549; Ward et al. [1994]=16,856), Scoles and Graves surveyed about 1.8% of the mtDNA genome, whereas we surveyed only about 0.3%. However, although it is of course true that had we surveyed more restriction enzymes, we would have uncovered many additional haplotypes, the two enzymes that we did select revealed most of the mtDNA diversity shown by Scoles and Graves (1993). For example, the (pooled) 12-enzyme haplotype diversity of 0.840 of Scoles and Graves was not much larger than our (pooled) 2-enzyme diversity of 0.677. Four of the enzymes used by Scoles and Graves showed no variation at all in the 120 fish and therefore were of no use for population discrimination. Twenty of the 34 12-enzyme haplotypes detected by Scoles and Graves (1993) among their 120 fish were seen only once, whereas only four of the 22 2-enzyme haplotypes in our 655 Atlantic and Pacific fish were seen only once: such rare haplotypes are of extremely limited use in population studies. Given that mtDNA heterogeneity among regions is very limited, it is not surprising that the approach of screening large numbers of fish for a small number of sequences known to be variable should be more powerful than screening small numbers of fish for a larger number of sequences, many of which are relatively invariant.

MtDNA data from another tuna, the albacore, *Thunnus alalunga*, showed a somewhat more pronounced separation of Atlantic Ocean and Pacific Ocean collections than did data for yellowfin tuna, but again no intraoceanic heterogeneity was detected (Chow and Ushiama, 1995).

The limited mtDNA differentiation among yellowfin tuna sampled throughout their range contrasts with the marked population subdivision revealed by the *GPI-A** locus. Mitochondrial DNA has an effective population size only one quarter that of nuclear DNA (Birky et al., 1989) and evolves more rapidly

(Brown et al., 1979); in principle it should be a more effective indicator of population substructure than nuclear loci. Given that more mtDNA than nuclear DNA divergence is expected, how can an allozyme locus show differentiation when mtDNA haplotypes do not? The lack of mtDNA differentiation in yellowfin tuna does not appear to be the result of a lack of variation nor of a small sample size: although increasing haplotype diversities and sample sizes will increase statistical power, the mtDNA haplotype diversities of our populations, assayed for just two restriction enzymes, were quite high at around 0.65–0.70, and sample sizes were similar to those used in the allozyme analyses. Nuclear DNA differentiation can exceed mtDNA differentiation when either the migration rate or the breeding sex ratio is strongly biased towards females (because mtDNA is maternally inherited), but there is no evidence that either of these conditions holds for yellowfin tuna (e.g. IATTC, 1992). The explanation for the seeming discrepancy may be that several independent polymorphic allozyme loci were screened, whereas haplotypes of mtDNA are best treated as alleles at a single, nonrecombining locus. In a situation of low overall genetic divergence (resulting from gene flow or recent separation), the stochastic nature of genetic drift means that if several allozyme loci are screened, and notwithstanding the expected higher rate of mtDNA evolution, divergence might be first detected at an allozyme locus before it is detected for mtDNA. An alternative explanation, as intimated earlier, is that the *GPI-A** differentiation results from selection.

The delineation of the four stocks of yellowfin tuna does not seem unreasonable given what we know of their distribution and movements. Yellowfin are found circumglobally, but only in tropical and subtropical oceanic waters, approximately between the latitudes 40°N and 40°S (Collette and Nauen, 1983). Spawning occurs throughout the year in all core areas of distribution, peaking in the warmer months (Collette and Nauen, 1983). Waters off the southern regions of South America (approximately 55°S) are too cold for Atlantic Ocean and Pacific Ocean fish to migrate around Cape Horn. Furthermore, direct connections between the tropical Atlantic Ocean and the eastern Pacific Ocean were severed after the Isthmus of Panama closed about 3.5 million years ago (e.g. Keigwin, 1982; Coates et al., 1992), a closure likely to have predated the origin of yellowfin tuna (estimated by Elliott and Ward (1995) to have occurred within the last two million years). Thus Pacific Ocean and Atlantic Ocean fish could not mix. In contrast, Atlantic Ocean and Indian Ocean fish could mix (through southern Africa waters), as could Indian Ocean and Pacific Ocean fish (through Indonesian waters), but tagging experiments indicate that most yellowfin tuna move on a scale of hundreds rather than thousands of kilometers (Joseph et al., 1964; Bayliff, 1979; Hunter et al., 1986; Lewis, 1992). The extent of migration between ocean basins is therefore likely to be low, with intraoceanic recruitment predominating. Nonetheless, interoceanic movements are possible and could account for the low degree of genetic differentiation among areas. Further discussion of the genetic and other biological data with respect to Pacific Ocean fish is given in Ward et al. (1994).

At present, these suggestions on the global stock structure of yellowfin tuna are essentially based on gene frequencies at a single polymorphic allozyme locus, *GPI-A**, because no significant genetic heterogeneity was detected for three other polymorphic allozymes and the mitochondrial DNA variants showed little interpopulation differentiation. It may well be that the stock structure of yellowfin tuna, in management terms, is more complex than these present findings suggest: very limited migration between areas can effectively homogenise gene frequencies, and thus dispersal between areas can still be low even between populations that cannot be genetically discriminated.

Future genetic work should include the examination of more fish from the Indian Ocean because the identification of these fish as a separate stock is based primarily on the analysis of just 21 fish for a single allozyme locus. Further clarification of genetic stock structure issues in yellowfin tuna will require larger sample sizes, examination of more areas (especially from the Indian and Atlantic Oceans), and the deployment of genetic techniques, such as microsatellite analysis, with enhanced resolving power and less concern over neutrality and selection issues.

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An estimate of orange roughy, *Hoplostethus atlanticus*, biomass using the daily fecundity reduction method

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Abstract.—The recruited biomass of orange roughy, *Hoplostethus atlanticus*, was estimated for the New Zealand mid-east coast orange roughy stock with the daily fecundity reduction method (DFRM). These fish migrate to Ritchie Bank and spawn between 850 and 900 m for about one month in winter. The biomass of spawning females was estimated by dividing mean daily planktonic egg production, N_0 (eggs/day), by mean daily fecundity, D (eggs/kg per day). The stock biomass was then estimated by multiplying the spawning female biomass by the ratio of all recruited fish to females that would spawn that year, estimated with a wide-area trawl survey made over the stock area two months before the spawning season.

The mean daily planktonic egg production was sampled near the peak of the spawning season, by using a stratified-random plankton survey. Eggs were staged and aged after accounting for their thermal history as they ascended the water column. Because young eggs were damaged by the net and older eggs were affected by advection out of the plankton survey area, relatively few egg stages were available for estimating N_0 (10.9×10^9 eggs/day), and the estimate was somewhat imprecise (CV=0.46). Mean daily fecundity (787 eggs/(kg × day), CV=0.11) was estimated from the daily rate of decline in population fecundity per mature female weight (R_i). Fecundity per female weight was estimated from a trawl survey made in the spawning area during the spawning season and was calculated as the mature eggs/kg of active spawners multiplied by the proportion of active spawners in each trawl. Spawning female biomass was 14,000 t (CV=0.50), and stock biomass was 26,000 t (CV=0.50). Mean daily fecundity was probably under-estimated because spent fish appeared to migrate from the spawning area during the fecundity reduction measurement period and reduce stock biomass to about 18,200 t. The DFRM biomass estimate was of central importance in the introduction of greatly reduced total allowable catch levels in this fishery.

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The orange roughy (*Hoplostethus atlanticus*, Trachichthyidae) fishery on Ritchie Bank on the eastern New Zealand continental slope (Fig. 1) was the second largest orange roughy fishery in New Zealand during the late 1980's and early 1990's, with a total allowable commercial catch (TACC) of about 10,000 metric tons (t) per year. Trends in catch per unit of effort (CPUE) indicated that the stock size was diminishing rapidly under this management regime, although the stock reduction analysis for the fishery did not estimate stock size precisely (Field et al.[1]). Experience with other major orange roughy fisheries in New Zealand (the "Box" fishery on northern Chatham Rise and the Challenger Plateau fishery [Fig. 1; Clark, 1995]) indicated that overfishing of the Ritchie Bank stock was likely because of low productivity. However, without adequate knowledge of stock size, it was difficult to set a TACC that would allow a sustainable fishery.

Zeldis (1993) concluded that both the annual egg-production method (AEPM; Saville, 1964; Picquelle and Megrey, 1993; Koslow et al., 1995) and the daily fecundity reduction method (DFRM; Lo et al., 1992; Lo et al., 1993) would be feasible for the estimation of absolute spawning biomass of orange roughy. A voyage was made to Ritchie Bank from early June to early July, 1993 (*Tangaroa* voyage TAN9306), with the intention of using both types of egg-production survey method. With the AEPM, annual egg production is the sum of daily planktonic egg production estimates made over the entire spawning season from separate subsurveys. Unfortunately, the voyage failed to sample annual egg production for two reasons. First, although the voyage was executed as a series of five subsurveys, two of the subsurveys were not completed because of ship and equipment breakdowns and because of a lack of time at the end of the voyage. Second, the voyage period ended before spawning had finished for the season, so that the annual egg production was not completely sampled.

[1] Field, K. D., R. I. C. C. Francis, and J. H. Annala. 1993. Assessment of the Cape Runaway to Banks Peninsula (ORH 2A, 2B, and 3A) orange roughy fishery for the 1993–94 fishing year. MAF Fisheries, Fisheries Assessment Research Document 93/8. NIWA, Greta Point, Wellington, New Zealand, 17 p.

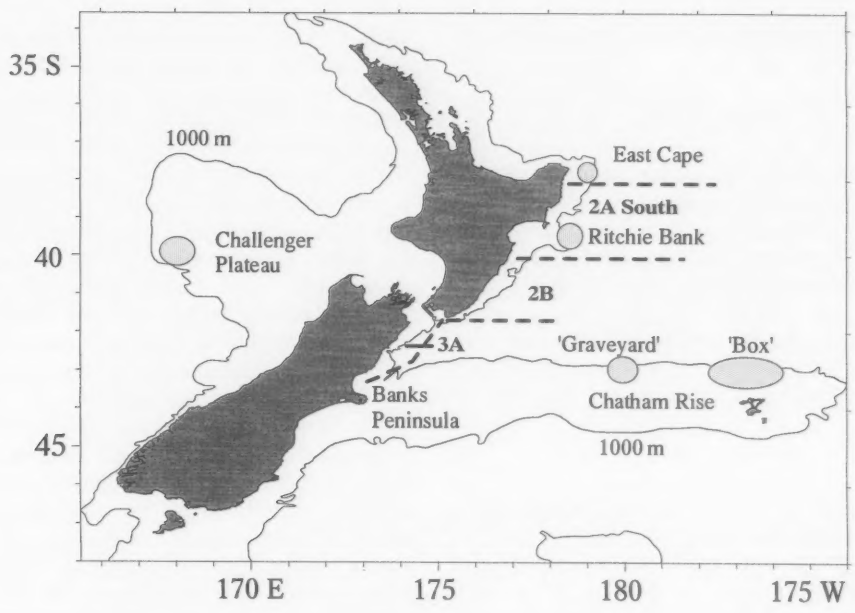

Figure 1

Map of New Zealand and location of spawning grounds (ovals) of orange roughy, *Hoplostethus atlanticus*. Also shown are the areas (2A, 3B, and 3A) of the east coast stock.

Unlike sampling for the AEPM, sampling for the DFRM did not need to cover the entire spawning season, allowing an estimate of spawning female biomass on Ritchie Bank to be produced from the portion of the voyage that did not suffer from ship and equipment breakdowns and that provided adequate data on planktonic egg production and fecundity. The DFRM was used to estimate the biomass of spawning females by dividing the daily planktonic egg production in the survey area (eggs/day) by the daily fecundity of females (eggs/(kg × day). The biomass of spawning females was scaled by the maturity ogive, sex ratio, and spawning proportion to estimate the recruited biomass (≥32 cm standard length) of orange roughy in the stock (where the stock is defined as those fish assumed to spawn on Ritchie Bank). The latter data were taken from a wide-area trawl survey in March–April 1993 (*Tangaroa* voyage TAN9303; Field et al.[2]), that covered the area inhabited by the stock (Banks Peninsula to East Cape, Fig. 1).

A necessary biological prerequisite for using the DFRM is that the target species has determinate annual fecundity (Hunter and Lo, 1993). This enables total seasonal fecundity to be determined before final maturation so that the fecundity reduction rate can be monitored through the spawning season (Lo et al., 1993). Orange roughy have determinate annual fecundity (Pankhurst et al., 1987; Bell et al., 1992; Zeldis, 1993). Application of egg production methods to orange roughy also is possible, but only if the age of planktonic eggs at morphological stage can be estimated. Ageing of eggs was achieved by developing a model for ageing the eggs as they traversed the thermal gradient in the water column (Zeldis et al., 1995) and by describing the morphological stages of the eggs (Grimes et al.[3]).

DFRM model

To calculate the biomass of Ritchie Bank spawning females, the daily planktonic egg production in the

[2] Field, K. D., R. I. C. C. Francis, J. R. Zeldis, and J. H. Annala. 1994. Assessment of the Cape Runaway to Banks Peninsula (ORH 2A, 2B, and 3A) orange roughy fishery for the 1994–95 fishing year. MAF Fisheries, Fisheries Assessment Research Document 94/20. NIWA, Greta Point, Wellington, New Zealand, 24 p.

[3] Grimes, P. J., A. C. Hart, and J. R. Zeldis. 1997. Embryology and early larval development of orange roughy (*Hoplostethus atlanticus* Collett). Unpubl. data.

survey area was divided by the daily fecundity/kg of the females:

$$B_{spf} = N_0/(1{,}000D),$$

where B_{spf} = biomass of spawning females (tons);
N_0 = daily egg production, (eggs/day);
D = mean daily fecundity (eggs/(kg × day)) for mature fish; and the factor 1,000 converts kg to tons.

The recruited biomass, B_{rec} (defined as the biomass of fish of length ≥32 cm) was calculated from the biomass of Ritchie Bank spawning females, B_{spf}, as

$$B_{rec} = B_{spf}S,$$

where the scalar (S) was an estimate of the ratio B_{rec}/B_{spf}. This ratio allows for recruited females that did not spawn, females that did spawn but were <32 cm, as well as the sex ratio.

To estimate the parameters of the above biomass model, the data analysis deals with three distinct data sets:

- daily planktonic egg production;
- daily fecundity per female weight; and
- proportions spawning, recruited, and female.

Daily planktonic egg production

Survey design The timing of plankton sampling coincided with the period when orange roughy females on the Ritchie Bank were in late maturation or spawning stages (mid-June to the end of the first week of July; Pankhurst, 1988). The location of plankton sampling was determined from research trawl catch rates (Fincham et al.[4]), which showed adult biomass to be highly aggregated on Ritchie Hill[5] (Fig. 2, A and B) at the northern end of Ritchie Bank. Ritchie Hill catch rates accounted for 84% of the relative orange roughy biomass over the Ritchie Bank survey area in July 1986 (Fincham et al.[4]). In plankton sampling during the spawning season on Ritchie Bank in early July 1986,[6] orange roughy eggs were caught only at stations near Ritchie Hill, and samples taken ≥20 km away contained no eggs, indicating that eggs were aggregated near the spawners (Zeldis, 1993) and that plankton sampling would need to be highly concentrated near Ritchie Hill.

During their first 36 hours of development, orange roughy eggs ascend the water column at 300–350 m/day from a spawning depth of about 850 m (Zeldis et al., 1995). Geostrophic currents over Ritchie Bank during July 1986[6] were to the south and averaged about 12 cm/sec between 700 m and 250 m; these currents would displace these eggs at least 15.5 km to the south of Ritchie Hill by the time the eggs had reached 36 h of development (this is a minimum estimate because there was probably some residual flow at the postulated level of no motion at 700 m). Considering that drift would probably vary in direction and speed but would lie predominantly along isobaths, we designed the survey area with eight strata, elongated alongshore and arranged symmetrically around a central stratum centred over the top of Ritchie Hill (Fig. 2B). This central stratum (10.0 × 7.6 km) was about the size of the area of high fish density observed during a trawl survey done in the area in June–July 1987 (Grimes[7]). The middle layer of four strata surrounding the central stratum had outer boundaries of 18.5 ×13.7 km. These dimensions were chosen to approximate the distance at which catch rates of 1-day-old orange roughy eggs on the St. Helen's seamount in eastern Tasmania were reduced to half (9.3 km from the spawners [Koslow[8]]). The outer layer of four strata had an outer boundary 40.0 × 30.0 km long, to allow for maximal drift of 36-h-old eggs.

The St. Helen's data were used to estimate optimal allocation of stations to Ritchie Hill strata. The St. Helen's data were collected over an entire spawning season, in six separate subsurveys, each of which had two spatial strata. The counts in each St. Helen's subsurvey and stratum were standardized such that their means equalled the mean across all of the subsurveys for each stratum. This procedure removed the within-season variation in mean egg density in each stratum. These standardized data were then laid over the Ritchie Bank stratum layers (central, middle, and outer), and mean egg densities (M_j) were estimated for each stratum layer, j. To allocate sta-

[4] Fincham, D. J., D. A. Banks, and P. J. McMillan. 1987. Orange roughy trawl survey, Tolaga Bay to Cape Turnagain, 14 June to 11 July 1976: cruise report. Fisheries Research Division Internal Report 60. NIWA, Greta Point, Wellington, New Zealand, 38 p.

[5] The names "Ritchie Bank," "Ritchie Hill," and "North Hill" used in this paper refer to the features called "Ritchie Ridge," "Calyptogena Bank," and "Pantin Bank," respectively, in the following: Arron, E. S., and K. B. Lewis. 1992. Mahia, 2nd ed. N.Z. Oceanogr. Inst. Chart, Coastal Series, 1:200,000. NIWA Greta Point, Wellington, New Zealand.

[6] Zeldis, J. R. 1986. Cruise report J08/86 (second half). MAF Fisheries unpublished cruise report held in NIWA Library, Greta Point, Wellington, New Zealand, 7 p.

[7] Grimes, P. 1987. NIWA, P.O. Box, 14-901, Kilbirnie, Wellington, New Zealand. Unpubl. data.

[8] Koslow, T. 1992. CSIRO Division of Fisheries, GPO Box 1538, Hobart, Tasmania 7001, Australia. Personal commun.

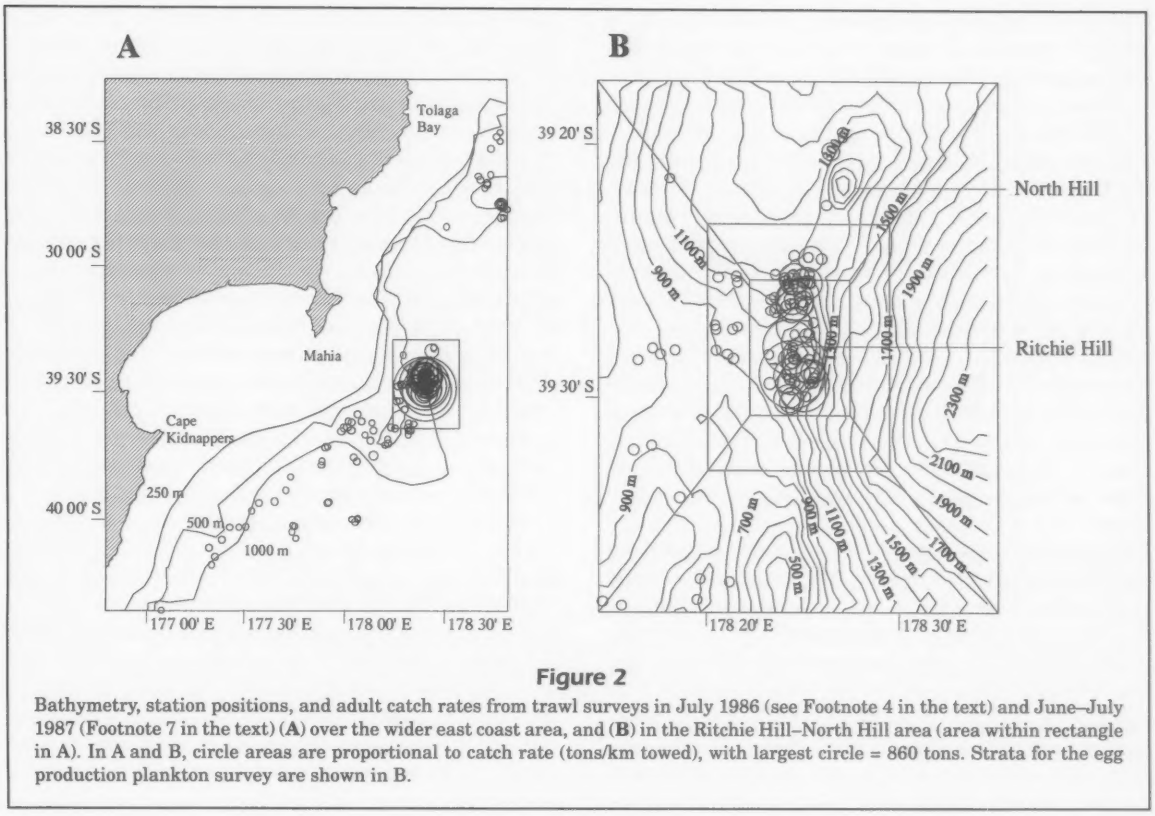

Figure 2

Bathymetry, station positions, and adult catch rates from trawl surveys in July 1986 (see Footnote 4 in the text) and June–July 1987 (Footnote 7 in the text) (**A**) over the wider east coast area, and (**B**) in the Ritchie Hill–North Hill area (area within rectangle in A). In A and B, circle areas are proportional to catch rate (tons/km towed), with largest circle = 860 tons. Strata for the egg production plankton survey are shown in B.

tions optimally in a survey of size N stations, n_j stations were allocated to each stratum layer such that

$$n_j \propto A_j M_j$$

and

$$\sum_j n_j = N,$$

where A_j = the area of each stratum layer.

Allocation was done in proportion to the strata means (M_j) because they were highly correlated with the strata standard deviations and were probably estimated more reliably than the standard deviations (Francis, 1984). To estimate values of N that would yield a desired coefficient of variation for egg abundance estimates, the standardized counts in each stratum layer were randomly sampled with replacement (bootstrapped) to estimate M_j, where the number of samples taken from each stratum was n_j. The survey mean egg abundance combined across all strata, E, was estimated as

$$E = \sum_j M_j A_j.$$

This procedure was repeated 500 times and the mean of the 500 survey estimates was taken as the egg abundance estimate. The standard deviation of the 500 estimates divided by the mean was taken as the coefficient of variation of the egg abundance estimate.

This analysis suggested that the optimal allocation would have stations allocated to the central, middle, and outer strata in ratios of 1.5:0.38:0.25, respectively. It also suggested that 400 stations would provide adequate precision (CV=0.15) in the egg abundance estimate. Therefore, for the AEPM design, five 80-station subsurveys were planned with stations allocated to strata in the above ratios.

Using simulations, we found that by occupying stations within each stratum in an order which minimized steaming distance between stations, before moving to a new, randomly chosen stratum, about 50% less steaming time would be involved than by occupying stations in completely random sequence in each subsurvey. This procedure would be done, however, at the cost of variable (and possibly long) periods of no coverage of each stratum between subsurveys and could be a serious drawback if spawning intensity varies significantly and rapidly (over a few days) during

the spawning season, especially for coverage of the central stratum. To counteract this, the stations in the central stratum were divided randomly between two time strata to reduce the time between occupation of this high-density stratum between subsurveys.

In the DFRM analysis, the plankton samples used for the estimation of egg production were from two consecutive AEPM subsurveys that were occupied near the peak of the spawning season and that were not subject to "downtime" from ship and equipment failures. These two subsurveys had no time break between them and were treated as a single survey, in which the central stratum was occupied four times and each of the surrounding strata was occupied twice. The plankton sampling in the entire survey was done from 14 June to 7 July, and the two subsurveys used in the DFRM analysis were done from 28 June to 6 July (subsurveys 3 and 4).

Egg sampling and staging, count standardization, and production estimation The plankton net used in sampling had a cylinder-cone design with 900-μm mesh, a mouth area of 2 m^2, and was fitted to a 125-kg flat-steel ring. It was designed to be efficient, with the ratio of the open area in the mesh to mouth area being >5:1 (Tranter and Smith, 1968). The net was deployed from a starboard crane while the ship was stationary (i.e. not under power) and its starboard side faced the wind. The winch had dynamic tensioning, to minimize surging of the net as a result of the rolling motion of the ship. Tow depths were within 30 m of the bottom to the surface if the bottom was less than 950 m and from 850 m if the bottom was deeper than 950 m. A conductivity-temperature-depth (CTD) probe or a net sonde within the mouth of the net was used to measure net depth. Warp payout was measured with winch instrumentation. Warp payout and recovery rates were 1 m/sec, also measured with winch instrumentation.

Eggs were staged (Grimes et al.[3]) on board, prior to preservation, and generally within 0.5 h of landing the net, except for two samples with many eggs. For these, staging was done partly on board and partly in the laboratory on 4% formaldehyde-preserved eggs. All eggs ≤ stage 7 (32-cell) were grouped, because it was not possible to identify with confidence the stages from germinal disk through 32 cells within the plankton samples because most of these younger eggs were damaged (76% of the 8,293 eggs ≤ stage 7), which caused the cell walls of the embryos to rupture, the cells to fuse, and the perivitelline space to collapse. Justification for assuming that the damaged eggs were all ≤ stage 7 are given in Zeldis et al. (1995) and Grimes et al.[3]

The standardization from egg count to egg density (eggs/m^2) was based on the formula *density* = *count* × *correction factor*, where the correction factor takes into account the mouth area of the net and the volume of water filtered by it. With a vertical haul, the correction factor is 0.5 (because the net mouth area=2 m^2). However, because the vessel almost always drifted (owing to wind and current) during shooting and hauling, hauls were not vertical and therefore the correction factors were almost always <0.5. Distance towed was not estimated by using flowmeters because flowmeters were found to record spurious revolutions during the deployment (descent) phases of tows in subsequent tests (Grimes[9]). Instead, to calculate the volume of water filtered by the net, it was necessary to use global positioning satellite (GPS) vessel positions, warp length, depth, and current velocities to infer the path of the net (which, because of the ship's drift, would be curved in the vertical dimension during hauling; Appendix 1).

With a curved net trajectory, there was a different correction factor for each combination of plankton tow and egg stage because the different egg stages occupied different depths as they ascended the water column and because the net sampled more water in layers of equal thickness in the upper water column than in the lower column. To calculate egg age and depth range (Table 1), data on egg development rate as a function of temperature, buoyancy by egg stage, and temperature as a function of depth were

[9] Grimes P. 1996. NIWA, P.O. Box 14-901, Kilbirnie, Wellington, New Zealand. Unpubl. data.

Table 1

Egg age (h) and depth (m) ranges used in calculating correction factors for count standardization for Ritchie Bank plankton samples. Egg stages are described in Grimes et al. (Footnote 3 in the text).

Stage	Min. age	Max. age	Max. depth	Min. depth
0	0.0	0.0	850	725
1	0.0	5.1	850	784
2	5.1	8.2	784	743
3	8.2	11.2	743	704
4	11.2	14.0	704	667
5	14.0	16.7	667	631
6	16.7	19.3	631	596
7	19.3	21.8	596	563
8	21.8	24.1	563	531
9	24.1	26.3	531	500
10	26.3	28.4	500	470
11	28.4	33.4	470	400
12	33.4	40.0	400	301
13	40.0	45.5	301	216
14	45.5	50.2	216	143
15	50.2	54.3	143	78

used with the methods of Zeldis et al. (1995) and the Ritchie Bank CTD temperature profiles described below. Ritchie Bank temperatures were within the range of those observed in Zeldis et al. (1995). Current velocities as a function of depth were calculated from geostrophic velocity profiles (Pond and Pickard, 1978) by using Guildline CTD profiles taken over Ritchie Bank. A reference depth of no motion at 1,000 m was assumed, and geostrophic velocities at 100 m were corrected to match the 100-m velocities measured by a buoy drogued to that depth. This buoy was deployed over Ritchie Hill on 28 June 1993, relocated 20 hours later, and subsequently lost.

Correction factors were to convert egg counts to densities were also calculated (assuming the trajectory of the net was straight) for comparison with those where a curved trajectory was assumed. For this, the volume filtered v (m^3) was estimated by using

$$v = 2\sqrt{p^2 + z^2},$$

where p = the distance the ship drifted from deployment to recovery of the net, determined with GPS;
z = the maximum depth of the net; and
factor 2 = the area (m^2) of the net mouth.

It was assumed that, at the start of hauling, the net was at the position of the vessel when deployment commenced, i.e. the net dropped vertically through the water during deployment.

Estimates of egg abundance by age group in the survey area (N_a) were calculated by multiplying the mean egg density at age in each stratum by stratum area and by summing across strata. In the case of the time strata in the center of the survey area, the egg abundances were averaged before summing with the other strata. The CV of N_a was calculated by using the standard deviation of egg density at age and stratum, weighted by stratum area. The average of the standard deviations was used in the case of the central time strata.

The maximum age of eggs that could be used in estimating daily egg production was the maximum age for which there appeared to be no significant advection out of the survey area owing to water movements (a loss of eggs by advection would cause a negative bias in N_a). Advection was examined by plotting centroids of each age group (Appendix 2) and by using the buoy and CTD data described above.

The daily egg production, N_0 (eggs/day), and instantaneous mortality for eggs, Z (per day), were estimated by maximum likelihood with the mortality model

$$N_a = N_0 e^{-zt},$$

where t = the mean age (days) of age group a (Appendix 3).

The precision of these estimates was estimated by a bootstrap procedure (Appendix 3).

Daily fecundity per female weight

Survey design Female fecundity and ovarian stage samples were taken from trawls from the RV *Tangaroa* on Ritchie Bank from 7 June–6 July and from commercial vessels on 22 June, 11 July, and 13 July (Table 2). Trawling was done during the week before spawning started (8–11 June), to sample total annual fecundity for the AEPM, and from the start of spawning until spent fish were common (20 June–13 July), to sample fecundity reduction for the DFRM. Twenty five of the trawls were from Ritchie Hill (within the area of the central stratum in Fig. 2B) and three were from North Hill, a spur off the north end of Ritchie Bank, about 12 km north of Ritchie Hill (Fig. 2B) where fish were spawning.

Oocyte sampling, ovarian staging, and daily fecundity estimation The oocytes of 569 mature fish (about 35 fish/trawl) in macroscopic ovarian stages 3 (late vitellogenic, prespawning), 4 (hydrated), 5 (ovulated), or 8 (late vitellogenic, partially spent) were counted. Of these, 218 fish were prespawning and used for total annual fecundity analysis. The remainder were used for DFRM analysis. Oocytes were counted by using the automated system described in Appendix 4.

The proportions of females in ovarian stages 3, 4, 5, and 8, and stage 6 (spent) in the trawl samples were estimated by using macroscopic ovarian staging of about 100 randomly chosen females per trawl.

The ovarian stages were further grouped because it was observed that stages 3 and 4 (group 1) had higher fecundities/ kg than stages 5 and 8 (group 2); this difference was due to fish in group 2 having started spawning. Therefore, the estimate of fecundity per female weight, R_i, was stratified by these groupings to minimize error. Stage-6 fish (spent) were placed in group 3. Thus, R_i was estimated as the mean number of eggs/kg of all females that would spawn, were spawning, or were spent, for trawl i:

$$R_i = \frac{\sum_{j=1}^{2} n_{ij} r_{ij}}{\sum_{j=1}^{3} n_{ij}}.$$

Table 2

Trawl stations used for estimation of total annual fecundity for the AEPM and fecundity reduction for the DFRM June–July 1993 on Ritchie Hill (R. Hill) and North Hill (N. Hill) (Fig. 2B). Catches taken on 22 June and 11 and 13 July were from commercial tows and catch sizes were unknown. No. staged = number of fish staged; Immat. = immature; Prop. active = proportion of active fish.

Date	Site	Catch (kg)	No. staged	Immat.	Stage 3	Stage 4	Stage 5	Stage 8	Stage 6	Prop. active
AEPM										
8 Jun	R.Hill	67	26	0	24	2	0	0	0	1.00
8 Jun	R.Hill	30	9	0	8	1	0	0	0	1.00
9 Jun	R.Hill	980	95	0	78	15	0	0	2	0.98
9 Jun	R.Hill	980	95	0	78	15	0	0	2	0.98
11 Jun	R.Hill	35	10	0	9	1	0	0	0	1.00
11 Jun	R.Hill	239	88	2	70	11	3	2	0	1.00
11 Jun	N.Hill	7,494	165	2	125	33	4	1	0	1.00
11 Jun	R.Hill	549	81	1	67	13	0	0	0	1.00
14 Jun	R.Hill	122	41	1	28	7	4	1	0	1.00
14 Jun	R.Hill	103	31	3	22	5	0	1	0	1.00
14 Jun	R.Hill	573	75	0	51	17	3	4	0	1.00
15 Jun	R.Hill	1,195	77	0	51	21	1	4	0	1.00
15 Jun	R.Hill	82	23	0	10	8	2	3	0	1.00
16 Jun	R.Hill	47	14	1	9	2	0	2	0	1.00
16 Jun	N.Hill	3,634	63	1	25	23	10	3	1	0.98
17 Jun	R.Hill	21,478	214	0	90	96	24	4	0	1.00
17 Jun	R.Hill	215	63	2	30	21	9	1	0	1.00
DFRM										
20 Jun	R.Hill	23,535	139	0	31	85	22	1	0	1.00
22 Jun	R.Hill	unknown	107	0	19	83	4	1	0	1.00
27 Jun	R.Hill	7,218	83	0	6	26	39	10	2	0.98
27 Jun	R.Hill	33,975	227	0	10	128	78	8	3	0.99
30 Jun	R.Hill	19,405	135	0	3	50	69	8	5	0.96
2 Jul	R.Hill	7,797	45	0	3	12	22	5	3	0.93
4 Jul	R.Hill	2,936	147	0	1	67	50	13	16	0.89
6 Jul	N.Hill	25,400	56	0	0	7	27	10	12	0.79
6 Jul	R.Hill	402	83	0	0	2	57	19	5	0.94
11 Jul	R.Hill	unknown	31	0	0	2	15	3	11	0.65
13 Jul	R.Hill	unknown	77	0	0	6	31	11	29	0.62

where n_{ij} = number of fish of ovarian group j at station i; and
r_{ij} = mean fecundity (eggs/kg) of fish of ovarian group j at station i.

The mean fecundities/kg of fish of ovarian groups 1 and 2 (r_{ij}) were estimated as

$$r_{ij} = \frac{\sum_{k=1}^{m_{ij}} e_{ijk}}{\sum_{k=1}^{m_{ij}} w_{ijk}},$$

where e_{ijk} = total fecundity of the kth fish of group j in the fecundity sample from station i (adjusted by the gonad wall proportions of total ovary weight in Appendix 4);
w_{ijk} = body weight (kg) of the kth fish of group j in the fecundity sample from station i; and
m_{ij} = number of fish of group j in the fecundity sample from station i.

The R_i's from the 11 DFRM trawls were fitted with a linear regression against time (weighted by the total number of fish in the ovarian-stage sample for each R_i) to estimate D, which is the mean daily fecundity (eggs/(kg × day)) for mature fish. The CV of D was estimated as the standard error of the slope of the regression, divided by the slope.

Biomass of spawning females

The biomass of spawning females B_{spf} was calculated by using the DFRM model given above. The CV of

B_{spf} was determined from the standard error of 1,000 estimates of B_{spf}, formed by dividing the 1,000 estimates of N_0 (Appendix 2) by 1,000 normally distributed estimates of D, formed from the mean and standard error of D.

Proportions spawning, recruited, and female

The scaling factors needed for converting the biomass estimate of Ritchie Bank spawning females to one for recruited fish (≥32 cm SL for both sexes) for the entire mid-east coast stock were estimated from the March–April 1993 wide-area east coast trawl survey (Field et al.[2]), instead of from the trawl data gathered at the time of the egg survey, for two reasons. First, not all recruited fish spawn each year (and thus may not migrate to Ritchie Hill). Second, a more precise estimate of sex ratio is available from the trawl survey than from the relatively few trawls carried out during spawning (when sex ratios are most variable; Zeldis, 1993). The trawls from the wide-area survey used for the scaling factors were those from over the entire mid-east coast survey area, from just north of Ritchie Bank, south to Banks Peninsula (Fig. 1; quota management areas 2A South, 2B, and 3A, respectively). This is the likely distribution of the stock that migrates to the Ritchie Bank to spawn.[10] No spawning orange roughy have been located in areas 2B or 3A, and genetic data show that orange roughy from these three areas cannot be separated, whereas they are genetically distinct from orange roughy on Chatham Rise (Fig. 1).

Stage-3 (late vitellogenic) females in each trawl in the 1993 wide-area trawl survey were assumed to be those that would spawn that year (Bell et al., 1992). Because ovaries of stage-3 females are indistinguishable macroscopically from ovaries of fish in which massive atresia has occurred (Bell et al., 1992), the macroscopic staging was checked by histological examination of about 20 stage-3 fish collected on each day of the 28-day trawl survey.

Recruited biomass

The scalar (S) was calculated as

$$\frac{\sum_i \left(P_{rec,i}\, C_i \frac{A_i}{n_i}\right)}{\sum_i \left(P_{spf,i}\, C_i \frac{A_i}{n_i}\right)},$$

where $P_{rec,i}$ and $P_{spf,i}$ = the proportions (by weight) of recruited fish and spawning (stage-3) females, respectively, in the catch from the ith trawl;

C_i = the catch rate (t/nmi) at the ith trawl;

A_i = the stratum area for the stratum containing the ith trawl; and

n_i = the number of trawls for the stratum containing the ith trawl.

The precision of the estimates of S was estimated by using the following bootstrap procedure. For each trawl, the triplet $(X_i, P_{rec,i}, P_{spf,i})$ was calculated, where $X_i = C_i A_i / n_i$. One thousand simulated data sets were generated by drawing triplets at random with replacement. Each simulated survey contained the same number of trawls as the area of the original trawl survey. For each simulated survey a bootstrap estimate of S was calculated as

$$\frac{\sum_i (P_{rec,i}\, X_i)}{\sum_i (P_{spf,i}\, X_i)},$$

The bootstrap estimates of S were used to calculate a CV for S.

The recruited biomass, B_{rec}, was calculated by using the DFRM biomass model given above. The bootstrap estimates for S were combined with the bootstrap estimates of B_{spf} to obtain a CV for B_{rec}.

Results

Daily planktonic egg production

Planktonic eggs were first captured in very low numbers on 15 June during subsurvey 1 (Fig. 3). Egg abundance remained low until the end of subsurvey 2 (25 June). This subsurvey was prolonged by ship and sampling-equipment breakdowns, and only the central strata, two of the middle strata, and none of the outer strata were sampled. The breakdowns prevented further sampling until the start of subsurvey 3 on 28 June, when large quantities of eggs were captured. Catches then decreased during subsurvey 4 (ending 6 July). Sampling of subsurveys 3 and 4 was completed. In subsurvey 5, only the central stratum and one middle stratum were occupied before the scheduled survey period ended on 7 July. Egg production continued at this time.

[10] Annala, J. H., and K. J. Sullivan (compilers). 1996. Report from the Fishery Assessment Plenary, April–May 1996: stock assessments and yield estimates, 308 p. Unpubl. report held in NIWA Library, Wellington, New Zealand.

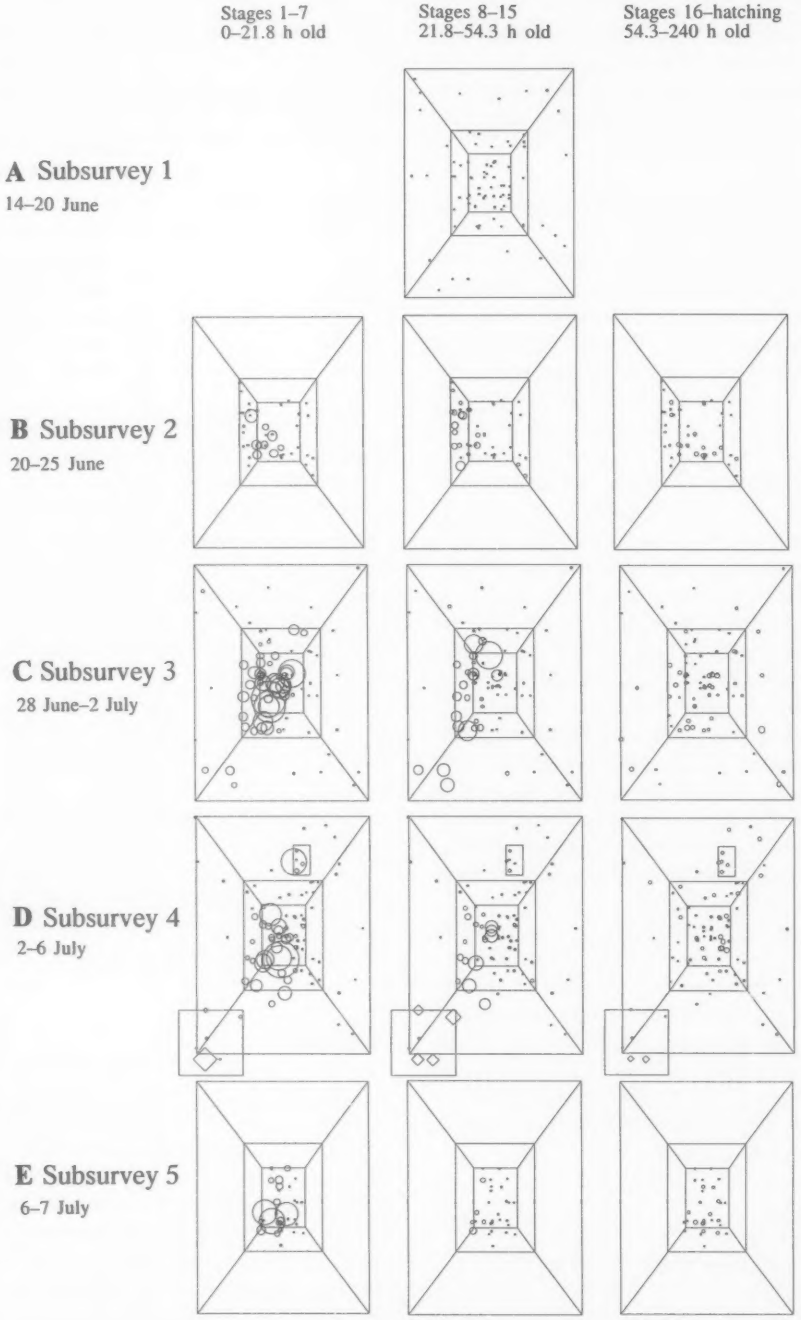

Figure 3

Positions and egg catch rates (m^2) for plankton tows by subsurvey and egg age group. In all panels (**A–E**), catch rates are proportional to circle area (zero catches are filled dots). Only six eggs were caught in subsurvey 1 (A); therefore only one panel was used to show station positions. The extra stations (diamonds) and strata were added after completion of subsurvey 4 shown in (D). The largest symbol in the figure = 310 eggs/m^2 sea surface area. Refer to Figure 2 for the plankton survey location and bathymetry.

Data from the completed subsurveys 3 and 4 showed that egg catches were highest in the central and middle strata (Fig. 3, C and D). These eggs were predominately in the young, grouped-age category (stage 7 or less, ≤21.8 h old; Table 1), but many middle-aged and some older eggs were also caught in these strata. A few orange roughy yolk-sac larvae were also caught in the central and middle strata. These high catch rates for eggs were spatially correlated with high research trawl catch rates for adults (Fig. 2, A and B; Table 2). Catches in the outer strata were usually <10 eggs/tow or zero eggs/tow. However, there was one large catch of young eggs near North Hill (Fig. 3D), on which relatively large catches of spawning adults were made with research trawling (Table 2) and commercial trawling. All of the undamaged eggs in this sample (19% of all eggs) were at the 1-cell stage, indicating that these eggs arose from localized spawning on North Hill. Four additional random tows were then made within an extra stratum created in this area (Fig. 3D) at the end of subsurvey 4. Only 5 additional eggs in total were caught in these tows, indicating that egg production on North Hill was low compared with that on Ritchie Hill.

A few moderate egg catches were also made in the southwestern corner of the survey area (Fig. 3, C and D). Most of these eggs (84%) were > stage 7. Four additional tows were then made within an extra stratum created in this area (Fig. 3D) at the end of subsurvey 4, and in three of these tows, all eggs were > stage 7. The fourth sample had many damaged eggs (≤ stage 7), but it was likely that these were at the older end of the age range of "young" eggs, judging by the stages of undamaged young eggs in that sample (all were ≥ stage 5). The bottom in the area where these samples were taken (Fig. 2B) is deeper (>1,000 m) than depths at which orange roughy normally spawn (850–900 m), and trawl catch rates in the area were very low in this survey and in previous surveys (Fig. 2, A and B). This finding indicated that these eggs had not been produced locally, but rather had been advected from the main spawning center on Ritchie Hill.

The positions of the centroids (centers of gravity) of successive egg age groups suggested that advection was initially to the southwest out of the survey area (Fig. 4A) but that older eggs (those of the very-broad-age-group stage 16+, ≥54.3 h old; Table 1) reentered the area, possibly from the east. In interpreting Figure 4, it is important to realize that centroids close to the boundary of the survey area were unlikely because only eggs from within the survey area were used in the calculation of centroid position. An estimate of the average rate of advection, at the depths of these egg stages, was calculated by dividing the distance between the centroids of stages ≤7 and 11 by the difference between the mean ages in these two age groups (Appendix 3) and was found to be 7.4 cm/sec.

The distribution of eggs by age within the survey area (Fig. 4, B and C) confirmed the southwesterly drift pattern. Eggs ≥ stage 11 (≥ 28.4 h old) became increasingly centered in the southwestern corner of the survey area (region 1, Fig. 4, B and C). However, the old eggs in the stage 16+ group were most abundant in the eastern and central regions (Fig. 4, B and C).

The advection inferred from egg distributions can be compared with hydrographic results. The drogued buoy was relocated 11.2 km south-southwest (bearing=207°) of the release site after 20 h at liberty (Fig. 5A), indicating that advection (at 100 m depth) was to the south-southwest, at a rate of 16 cm/sec. Geostrophic analysis of the CTD data (Fig. 5, A–D) indicated that in the northern part of the station grid (in the vicinity of the Ritchie Hill spawning site), current directions turned from south-southeast through south to southwest as depth increased from 100 through 400 to 800 m. The southwestern component of current velocity between 800 and 400 m (the approximate depth range of eggs ≤ stage 11; Table 1) in the vicinity of Ritchie Hill averaged about 7 cm/sec (Fig. 5D). Geostrophic current speeds averaged about 12–13 cm/sec in the upper 100 m of the water column, in the vicinity of Ritchie Hill. These speeds were probably underestimates because the velocity profiles showed little evidence of reaching asymptotically low values as the postulated level of no motion (1,000 m) was approached (Fig. 5D), suggesting that some residual velocity existed at that level. This may explain the greater buoy speed than geostrophic speed at 100 m. Current speeds toward the southwest (Fig. 5D) were higher on the northern side of the grid than on the southern side through the upper water column; a significant easterly component was observed in the upper 200 m.

Thus, the advection pattern indicated by egg centroids and stage distributions (Fig. 4, A and C) was consistent with results from the drogued buoy (Fig. 5A) and the geostrophic analysis (Fig. 5, A–D), which indicated that the direction of drift of young eggs in the lower half of the water column was toward the southwest. The geostrophic velocities in the lower half of the water column in the vicinity of Ritchie Hill (at least 7 cm/sec) were consistent with the velocity of egg advection (7.4 cm/sec) from our calculations. The older eggs, which would have spent most of their time in the mixed layer (Zeldis et al., 1995), might have been conveyed into the survey area from

the east by a return flow in the upper water column to the south and east of Ritchie Hill.

Egg abundances over the survey area during subsurveys 3 and 4 were calculated by using the curved and straight trajectory assumptions (Table 3). The ratios of the curved and straight abundances generally decreased with egg stage. This decrease was expected because the curved trajectory is nearer to vertical deeper in the water column (where the younger eggs are) than shallower in the column (where the older eggs are). The curved and straight trajectories tended to be nearly parallel in shallower water.

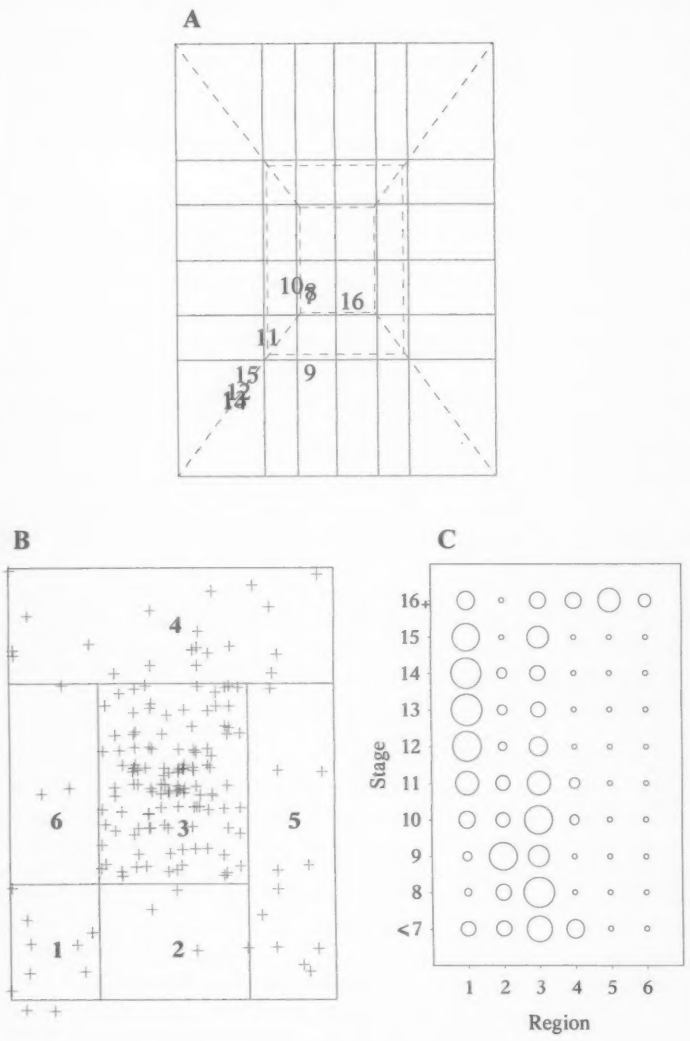

Figure 4

Studies of egg-stage distributions derived from subsurveys 3 and 4. (**A**) The positions of centroids of the egg stages and the 36 subareas of the plankton survey area (solid lines) used in calculating centroid positions (Appendix 2). Centroid 7 represents the combined stage group 1–7, and centroid 16 represents stages 16 to 29 (hatching). The stratum boundaries of the survey area (dotted lines, slightly offset) are shown for reference. (**B**) Six regions and stations used in the distribution analysis (region 3 comprised the inner and middle strata of the survey area), including the extra stations allocated in the southwestern corner. These latter stations were assumed to lie within region 1 for this analysis. (**C**) Proportions (circle areas) of eggs within the regions in B, by egg stage.

Because eggs were advecting into the southwestern corner of the survey area (region 1, Fig. 4C) by the time they reached stage 11, only stages ≤10 were used in the estimation of daily egg production. The criterion used for this cutoff stage was that all stages ≥ the first stage to have >20% of their abundance in region 1 would be excluded from the analysis. This criterion was reached at stage 11. Using the abundance data calculated by assuming a curved net trajectory (Table 3), we estimated that egg production was $N_0 = 10.9 \times 10^9$ eggs/day (CV=0.46) and that the mortality-rate estimate was Z = 0.70/day (CV=0.69) (Fig. 6; Table 4). These calculations used the extra strata at North Hill and in the southwest, and all stations in subsurveys 3 and 4 falling within these strata were considered to have been originally selected within them. The N_0 estimated by assuming a straight trajectory was 8.0×10^9 eggs/day (CV=0.49) with $Z = 0.56$ (CV=0.88). In the remaining analyses, the N_0 value calculated by assuming a curved trajectory was used because this value was likely to be a better approximation to the truth than that obtained by assuming a straight trajectory.

Daily fecundity of females

The proportions of females in each ovarian stage in each DFRM trawl (Fig. 7) showed that the female population was, at first, nearly all in the prespawning state (stage 3). Maxima of hydrated, ovulated, and

spent proportions followed at approximately 10–15 day intervals. Serial spawning was evident in that partially spent, hydrated, and ovulated proportions became fairly constant during a 10-day period (roughly 25 June–5 July) when ovaries of the fish were developing among these stages. Hydration did not appear to be associated with imminent spawning, because significant proportions (0.15) of hydrated fish were present about 5 days before planktonic eggs were first caught on 15 June. However, the first appearance of significant proportions (>0.05) of ovulated fish (14 June) and planktonic eggs (15 June) nearly coincided.

The decline in R_i (Fig. 8) or the daily fecundity per female weight, D, was 787 eggs/(kg × day) (CV= 0.11; Table 4).

Ritchie Bank spawning female biomass

When N_0 was divided by D, the estimate of B_{spf} was 14,000 tons (Table 4), with CV = 0.50.

Biomass of recruited orange roughy in the mid-east coast stock

The factor S was estimated to be 1.77, with CV = 0.03. However, histology showed that for 4.5% of the females identified as spawners in the wide area trawl survey, their entire exogenous vitellogenic oocyte complement was actually in the process of atresia (Bell et al., 1992). These fish were indistinguishable macroscopically from fish that would spawn successfully and did not appear to cluster in any particular area of the trawl survey. The factor S was scaled upward to account for these fish, resulting in S = 1.85 (Table 4).

The resulting estimate of B_{rec} was 26,000 t (Table 4), with CV = 0.50. The bootstrap procedure used may have slightly overestimated the CV of S because it did not fully take into account the stratum structure of the wide-area survey. However, this overestimation is of little importance because the CV of S was so much smaller than that of the other components that made up the CV of B_{rec} (Table 4).

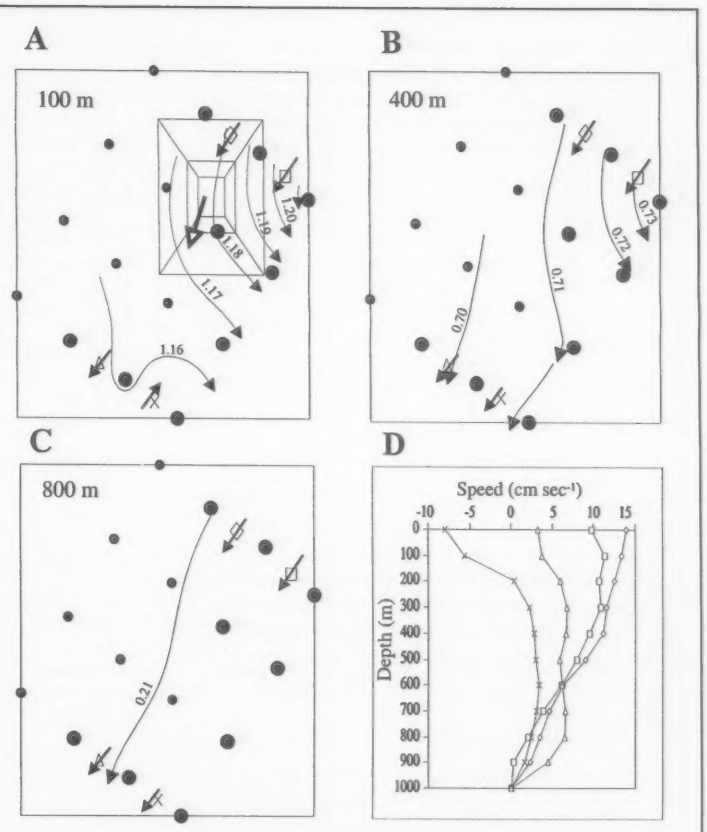

Figure 5

Contours of dynamic height (dynamic meters) at 100, 400, and 800 m in the hydrographic survey area (**A–C**). The arrows on the contours show inferred current directions. The large filled circles indicate stations used in the analysis (where cast depths were ≥ the 1,000 m reference depth). Panel (**D**) shows depth profiles of current velocity (cm/sec) at the symbols in A–C, flowing in the direction of the arrows through each symbol. In A, the plankton survey area is shown for reference and the start and end positions of the buoy deployment are shown by the beginning and end of the open-headed arrow.

Bias due to turnover

An important potential bias in the DFRM arises from the fact that the method is sensitive to turnover of fish on the spawning ground. For example, if fish arrive, complete spawning, and leave the trawl survey area *before* or *after* the trawl survey period, fecundity will be undetected and biomass will be underestimated. These effects were unlikely, however. Almost no eggs were caught in the first subsurvey (Fig. 3A), and very few spent fish were detected in trawls on Ritchie Hill until 2 July (Fig. 7). This finding suggested that no spawning was completed before the trawl survey began (8 June). In addition,

Table 3

Abundances of eggs ($\times 10^{-6}$) and coefficients of variation (CV) at stage, during DFRM planktonic egg survey, calculated with curved and straight net trajectory assumptions. Also given are the ratios of curved and straight abundances.

Stage	Curved abund.	CV	Straight abund.	CV	Ratios
≤7	7,036	0.26	5,453	0.28	1.29
8	487	0.40	424	0.43	1.15
9	673	0.60	451	0.51	1.49
10	316	0.43	276	0.49	1.15
11	400	0.24	351	0.26	1.14
12	1,420	0.32	1,276	0.32	1.11
13	740	0.23	716	0.24	1.03
14	276	0.38	260	0.37	1.06
15	34	0.42	38	0.38	0.90

proportions of macroscopic stage-3 (vitellogenic) fish declined to 5% by the midpoint of the trawl survey period and to 0% by the end; stage-4 (hydrated) fish showed a similar decreasing trend. Thus no prespawning fish were present at the end of the trawl survey (13 July).

Turnover during the trawl survey period may have biased the biomass estimate if prespawning fish arrived late to the trawl survey area (after trawling for R_i had started), at the beginning of the season. This means that not all prespawning fish would have been sampled by trawls in the spawning area, which would cause an underestimate of R_i, because fish that had started spawning would be over-represented. Similarly, if spent fish departed the trawl survey area early (before trawling for R_i had ended) toward the end of the season, spent fish would be under-represented by trawls in the spawning area. In this case, R_i would be overestimated because fish which had not finished spawning would be over-represented. Both of these effects (late arrivals and early departures) would cause an underestimate of D, which, in turn, would cause an overestimate of biomass, N_0/D.

Was it likely that late arrivals or early departures (or both) of spawners occurred in the present study? The annual fecundity/kg of prespawners, estimated from fish sampled before the R_i sampling period and before any eggs were caught in the plankton (Fig. 8), was 27, 271 eggs/(kg × yr). If this estimate is divided by the estimated daily fecundity/kg (787 eggs/(kg × day); Table 4), the period required for the average fish to spawn completely is 35 days. However, the time lag between the first appearance of significant proportions (>0.05) of ovulated and spent fish was about 19 days (from 14 June to 2 July; Fig. 7). If this lag is interpreted as the duration of spawning in individual fish, D was underestimated by the fecundity reduction trawling.

It would appear, however, that late arrival of prespawners to the trawling area did not contribute greatly to the underestimation of D. The R_i sampling period began on 20 June when prespawner (stage-3) proportions had become low (0.20; Fig. 7) and were decreasing rapidly. At this time the estimated R_i was only about 10% below the prespawning level (Fig. 8). Eggs first appeared in the plankton on 15 June, but catches of young eggs in the central strata were still relatively small (3.9 eggs/m^2; Table 5) during 20–25 June (subsurvey 2). Therefore, only a relatively small reduction in R_i would have been expected by

Table 4

Parameter estimates for DFRM for Ritchie Hill spawning female biomass and mid-east coast recruited biomass, June–July 1993 (with coefficients of variation in parentheses). N_0 = daily egg production for Ritchie Hill survey area (estimated with curved net trajectory); D = weight specific daily fecundity of females; B_{spf} = biomass of spawning females in Ritchie Hill survey area; S = ratio of recruited biomass to that of spawning females; B_{rec} = biomass of recruited fish. Parameter estimates with subscripts marked "*turn*" have turnover incorporated (see text); CV's were not estimated in these cases.

Parameter	Estimate
N_0	10.9×10^9 eggs/day (0.46)
D	787 eggs/(kg × day) (0.11)
B_{spf}	14,000 t (0.50)
S	1.85 (0.03)
B_{rec}	26,000 (0.50)
D_{turn}	1,106 eggs/(kg × day)
$B_{spf,\,turn}$	9,900 t
$B_{rec,turn}$	18,200 t

Table 5

Mean abundances (per m^2) of all eggs ≤ stage 7 and dates of sampling in the central strata for each subsurvey.

Subsurvey	Date	Mean	CV
1	15–17 June	0.0	0.0
2	20–25 June	3.9	0.34
3	29 June–1 July	49.0	0.29
4	3–5 July	22.6	0.46
5	6–7 July	22.2	0.50
4 and 5	3–7 July	22.4	0.34

20 June, in agreement with the reduction actually observed by that date (Fig. 8).

It was likely, however, that early departures caused spent fish to be under-represented in the trawl samples toward the end of the R_i time series. The abundance of young planktonic eggs in the central strata was reduced by more than half between subsurvey 3 (mid-date 30 June) and subsurvey 4 (mid-date 4 July; Table 5). Because subsurvey 3 was done when the spent proportion was virtually zero (Fig. 7), this reduction of planktonic egg abundance implied that about half of the fish had ceased spawning by 4 July (assuming that the spawning rate of remaining active fish was constant). However, only 0.11 of fish were recorded as spent on 4 July (Table 2; Fig. 7); thus this proportion appeared to be underestimated by 0.50 – 0.11 = 0.39 on 4 July. Commercial catch rates on Ritchie Hill (Fig. 9) also decreased considerably during the last week of June and first week of July, suggesting that fish abundance in the trawling area had declined.

To account for the error in D that the underestimation of spent fish would cause, the proportion of active fish on 4 July was adjusted downward to 0.89 – 0.39 = 0.50 to estimate a new D value of 1,106 eggs/(kg × day) (assuming a linear decline between 20 June and 4 July; Fig. 8; Table 4). This adjustment resulted in a period of 25 days for an average fish to spawn completely, which is not greatly different from the 19 days estimated from the time lag between ovulated and spent fish proportions (shown above). This re-estimation of D, to account for turnover, had proportional effects on B_{spf} and B_{rec} (Table 4).

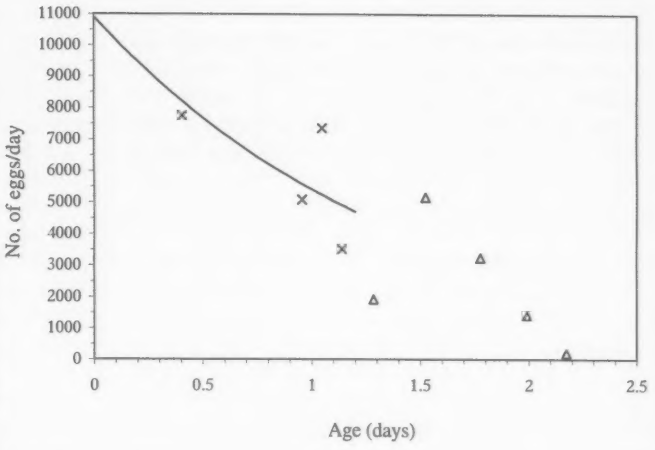

Figure 6

The daily production of eggs as a function of age, calculated by dividing the curved trajectory egg-abundance estimates (Table 3) by the duration of each age group and by plotting against the mean age of eggs in each age group (Appendix 3). The line was fitted by using N_0 and Z (Appendix 3), calculated with only egg stages ≤ 7 to 10 (crosses). Egg stages 11 to 15 (triangles), were not used in the production estimate because they were subject to advection out of the survey area (see text).

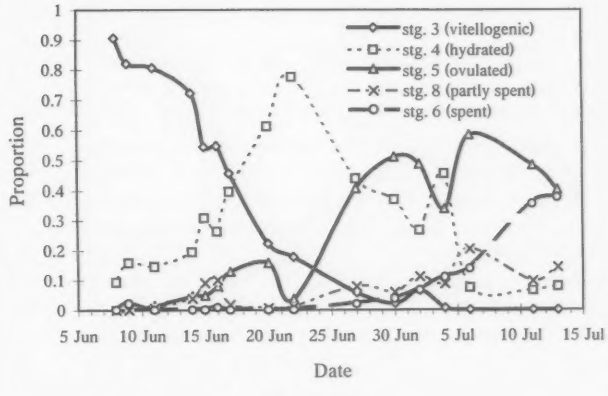

Figure 7

Time series of macroscopic ovarian stages over the survey period. Each point is the proportion of females at that stage, as a proportion of all mature females, averaged for all trawls on that date. Only trawls on Ritchie and North Hills were used (Table 2).

Discussion

This study has used the DFRM to estimate the biomass of recruited individuals in the mid-east coast orange roughy stock, by combining estimates of daily egg production rate, the rate of fecundity reduction, and the proportion of the stock that was spawning females. In this section various sources of error are discussed, and the methods and results of this study are compared with those of other studies. Also, a brief description is made of how the results from this survey have been used in assessing the stock.

Sources of error

In egg-production surveys, the trajectory of the plankton net during deployment and retrieval is usually assumed to be straight (and often, vertical). The analyses presented here (Appendix 1) suggest that, for the present survey, 1) there was significant horizontal movement of the net during deployment owing to drag from the warp (which was caused by ship drift), and 2) the plankton net followed a curved trajectory during hauling. The curved trajectory meant that less water was filtered in deeper than in shallower ocean layers of equal thickness and that larger correction factors were required for younger (deeper) eggs in order to standardize the egg counts to eggs/m^2. The effect of using curved, rather than straight, trajectories was to increase the estimated production rate by 36% (from 8.0 to 10.9 billion eggs/day). Recent egg-production survey work, using a digitally recording flowmeter system developed at NIWA (Grimes[9]), has shown that 1) there often are spurious revolutions of the flowmeter on the downcast, negating the use of conventional flowmeters and that 2) more water is filtered at shallower depths than at deeper depths, justifying the assumption of a curved net trajectory. Therefore, it is likely that by allowing depth variation in the estimates of the amount of water filtered, a major improvement was made over the assumption of a straight net trajectory.

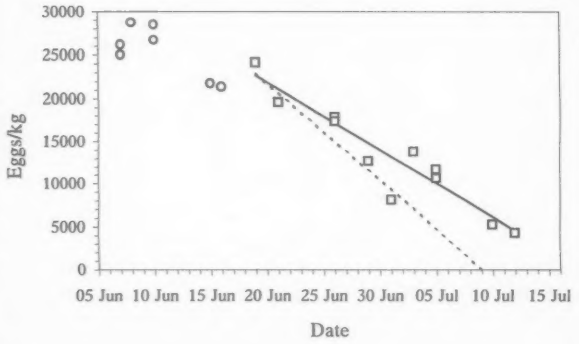

Figure 8

Weight-specific fecundity (eggs/kg spawning female: R_i) from trawls prior and during the DFRM sampling period (circles and squares, respectively, Table 2). Solid line is a weighted linear regression fitted for DFRM trawls. Dotted line is a regression fitted when it was assumed that proportion active was overestimated by 39% on 4 July (see text).

The precision of the planktonic egg production estimate was influenced by damage to early stage eggs (Zeldis et al., 1995; Grimes et al.[3]). In the present study, the inability to stage most eggs less than 21.8 h old caused a greater reliance on older egg stages to estimate production. However, the number of older egg stages that could be used for the estimation was limited because eggs older than stage 10 (28.4 h) were subjected to advection toward and through the southwestern boundary of the survey area as they aged. Thus, the original strategy of making the survey area large enough to retain all of the eggs up to 36 h old was defeated, and relatively few stages were available for a mortality estimate. Clearly, the combination of nearly concurrent information on ocean circulation (from CTD and drogued buoy) and on the drift patterns of the eggs themselves provided useful corroborative information for quantitative decisions about the egg-age range available for mortality estimation, when eggs were subject to advection.

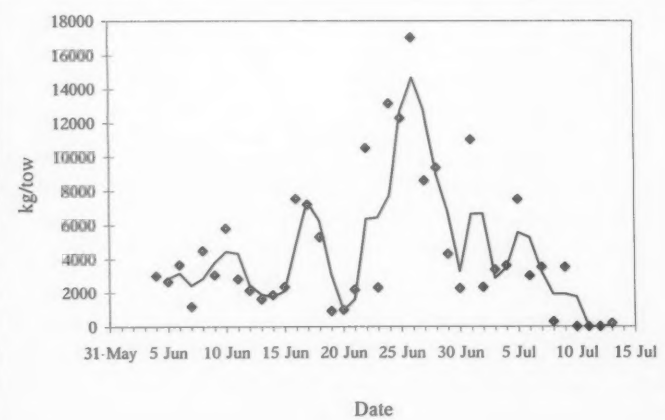

Figure 9

Commercial catch rates of orange roughy, *Hoplostethus atlanticus*, on Ritchie and North Hills, 4 June–13 July 1993. Points are the means of all catches by all vessels on each day (kg/tow), fitted with a moving average (line) with a 2-day period.

Precision estimates for the egg-abundance data may have been biased by potential autocorrelation among the data, which would not bias the esti-

mate of egg production but could cause its precision to be overestimated. No attempt was made to correct for this bias because the data were judged to be inadequate to estimate an autocorrelation structure (which would need to have both spatial and temporal terms). Picquelle and Megrey (1993) drew the same conclusion for pollock egg surveys. For the present study, a minimum spacing of 1,000 m was imposed when allocating the stations to the strata. This spacing should have gone some way toward minimizing the effects of spatial autocorrelation. In an explicit study of autocorrelation in anchovy egg catches, Smith and Hewitt (1985) found that spatial autocorrelation diminished rapidly at spatial scales of 2,000 m and that temporal autocorrelation diminished at 0.5 h for 1-day-old eggs. Given that the minimum distances and times between samples for the egg stages used in this study were of this order and that the egg catch rates were highly variable, overestimation of precision was likely to be minor.

Use of the DFRM required the assumption that the 11 trawls used to estimate D representatively sampled spawning females on Ritchie and North Hills. Although it is clear that spawning female biomass is highly aggregated in the Ritchie Hill area (Fig. 2), it is not known to what extent females are randomly distributed within this small area, with respect to ovarian stage. Therefore, it was useful to look at a much larger set of fecundity data collected on these hills in 1995 by N.Z. Ministry of Fisheries (MOF) scientific observers working on commercial vessels (Zeldis, unpubl. data). Samples were taken from 47 trawls made with four vessels (range of 7–16 trawls per vessel) throughout the entire spawning season. These tows were long, typically traversing much of the ridge between North Hill and the south side of Ritchie Hill (Fig. 2) and covering the 850–900 m depth range of orange roughy spawning. The fecundity reduction rate from these 1995 data (uncorrected for turnover) was 965 eggs/(kg × day) (CV=0.11), which was not significantly different from the uncorrected estimate from 1993 in the present study, 787 eggs/(kg × day) (CV=0.11; Table 4). This rate showed that widespread and intensive trawling in this area would yield an estimate of D similar to that from the less intensive research trawling of the present study, indicating that the less intensive trawling accurately represented the spawning dynamics of the population.

The reliance of the DFRM on within-spawning season trawl data makes it susceptible to bias due to turnover, which causes an underestimate of fecundity reduction rate. In the present study, it appeared that spent females left the survey area during the period when fecundity reduction was measured, such that only 0.11 of remaining females were spent 21 days after the start of spawning. A similar pattern of low proportion spent was seen in the 1995 Ritchie Bank scientific observer data described above, with proportion spent < 0.20 about 23 days after the onset of spawning. In contrast, in recent NIWA orange roughy egg production surveys done at East Cape in 1995[10] and at the "Graveyard" area on northern Chatham Rise in 1996[11] (Fig. 1), spent proportions were between 0.60 and 0.70 about 20 days after the onset of spawning. This suggested that turnover was less prevalent at these latter sites, an effect which may be related to the fact that although commercial fishing was intensive on Ritchie Bank during the 1993 and 1995 spawning seasons, there was no commercial fishing during the 1995 and 1996 spawning seasons at East Cape and the "Graveyard." Significantly, the value of D at East Cape was 1,036 eggs/(kg × day), with no correction for turnover, which was similar to the turnover-corrected value for the present study at Ritchie Bank of 1,106 eggs(kg × day) (an estimate is not yet available for the "Graveyard"). For this reason, the turnover-corrected fecundity reduction rate estimated for Ritchie Bank in 1993 is considered to be more reliable than the uncorrected estimate and also a reasonably reliable estimate of the true rate of decline in Ritchie Bank population seasonal fecundity.

Comparision with other studies

The first use of the DFRM was by Lo et al. (1992; 1993) to estimate the biomass of a deepwater pleuronectid flatfish, Dover sole (*Microstomus pacificus*), which spawns between 600 and 1,500 m depth on the continental slope of western North America. The model used in the present study to describe the daily fecundity reduction was simpler than that of Lo et al. (1993). It assumed that the fecundity per fish weight was a linear function of time only (fish weight was considered as an additional predictor but did not significantly improve the fit). Lo et al. (1993) assumed that both total fecundity of active females and the fraction of active females (their E_t and G_t) were linear functions of time and fish weight. The former model was used for two reasons. First, in calculating fecundity reduction, there seemed no need to treat active and inactive females separately. Second, in the absence of any evidence of lack of fit, the rule of Occam's razor suggested using the simpler model.

[11] Grimes, P. J. 1996. Voyage Report, TAN9608 (Part II). NIWA unpublished voyage report held in NIWA Library, Greta Point, Wellington, New Zealand, 4 p.

A second difference between the DFRM used here and that of Lo et al. (1993) is the way sex ratio and active female proportion were estimated and brought into the biomass model. In the present study, the proportion of all recruited fish that were spawning females was estimated with parameter S, whereas this proportion was estimated within the R and D parameters in the model of Lo et al. (1993). Orange roughy spawning takes place in dense aggregations that form after the spawners have migrated hundreds of km from the nonspawners. Therefore, it is not possible to estimate the proportion of active females to all recruited fish with the trawls done on the spawning ground during the spawning season (which are used to monitor fecundity reduction). This estimation must be done with a separate trawl survey over the whole stock area, preferably before the spawners have aggregated significantly. In contrast, Dover sole spawning appears to be dispersed over a wide latitudinal area of the North American west coast slope (Lo et al., 1993) and takes place over a long spawning season (6 months; Hunter et al., 1992). In Lo et al. (1993), the spawners were assumed to be dispersed in the same geographic region as the nonspawners during spawning, and therefore sex ratio and proportion active components were estimated from the same trawls that were used to estimate fecundity reduction.

There are few other estimates of planktonic egg mortality (Z) in the literature for deepwater spawners that can be compared with the value (Z=0.7) obtained for orange roughy in this study. Lo et al. (1993) fitted a Pareto decay function to Dover sole egg abundances, in which mortality was allowed to decrease with egg age. Initial mortality was 0.63 which halved by the age of 1 day. Another western North American slope species, sablefish (*Anoplopoma fimbria*), has been the subject of egg-production studies (Moser et al., 1994) and for this species, Z varied between 0.25 and 0.48, depending on region (it should be noted that counts of 1-day-old and 2-day-old eggs were excluded because these eggs were undersampled). Thus, the few Z estimates that exist for other deepwater species indicate variable mortality rates, but that mortalities can be quite high. Orange roughy egg abundance was estimated by Koslow et al. (1995) in their application of the AEPM to orange roughy biomass estimation on St. Helen's Seamount, Tasmania. These authors assumed that mortality in the first 28 h after spawning was zero and that the mean abundance of 0 to 28 h old eggs equalled N_0. This assumption was based on their finding no differences in abundance among fertilization or 1-cell eggs (stages 1 and 2 of the present study) and their subsequent two stages which were 2 cell and 4–128 cells (stages 3 and 4–9, respectively, of the present study; see also Grimes et al.[3]). As mentioned, the differentiation of stages 1–7 was not possible for the great majority of the samples in the 1993 Ritchie Bank survey because of egg damage. However, the probability that the mortality rate estimated from the grouped stages 1–7 and stages 8, 9, and 10 was zero was low (P=0.10).

The procedure used to estimate S, for converting spawning female biomass to recruited biomass, is easily adapted to estimate the proportion of females in the stock area that will spawn in the current year. For the mid-east coast stock in 1993, S was estimated as 0.52 of all mature females (by weight), a result similar to that for orange roughy stocks in southern Australia (0.45, by numbers; Bell et al., 1992). Wide-area trawl surveys of the east coast were also done in March–April of 1992 and 1994,[10] and these yielded similar values for these proportions (0.49 and 0.42 by weight). Also, a low proportion of females with fully atretic ovaries was found (0.045) over the mid-east coast survey area in 1993, again in common with the results of Bell et al. (1992).

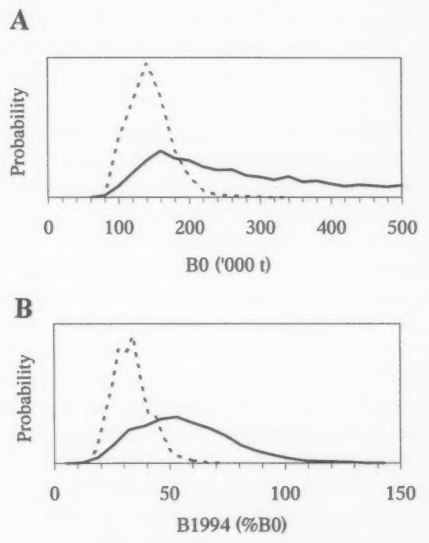

Figure 10

Probability distributions of (**A**) virgin biomass (B_0) and (**B**)1994 biomass as a percentage of B_0, from the 1994 stock reduction analysis for the mid-east coast orange roughy, *Hoplostethus atlanticus*, fishery (see Footnote 2 in the text). Each panel shows (solid line) the distribution when the analysis was done without the DFRM estimate (with only CPUE, trawl survey indices, and mean fish length data) and (dotted line) the distribution when the DFRM estimate was included.

Use in stock assessment

Although the biomass estimates derived from this DFRM survey were rather imprecise (CV=0.50), they were capable of having a dramatic effect on the assessment of the mid-east coast stock. In 1994, an estimate of 45,000 t recruited biomass (CV=0.40) from a preliminary analysis of these data was used in assessing this stock (Field et al.[2]: this estimate differs from those given above because some data errors have subsequently been corrected and the analyses have been refined). The inclusion of this estimate greatly improved the precision of the assessment (Fig. 10), which resulted in a 2-year stepped reduction in TACC from 10,333 t in 1993–94 to 2500 t in 1995–96.

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Appendix 1: Calculation of "curved" correction factors

This appendix describes, for the case where the path of the net during hauling is assumed to be curved, the calculation of the correction factors that convert egg counts to egg density (eggs/m^2).

For each plankton tow, we calculated the following:

1 the position of the net at the start of hauling;
2 the position of the net at a series of equally spaced times during hauling;
3 the flow of water through the net at each of these times; and
4 a correction factor for each of these times.

Finally, interpolation was used to calculate the correction factor when the net was at the mid-point of the depth layer associated with each egg stage. This was taken as the correction factor for that egg stage at that plankton tow.

First, the assumptions behind these calculations and some notation are defined.

Assumptions and notation

The calculations required the following assumptions:

1 the vessel drifted at a constant velocity while shooting and hauling;
2 the net dropped at a constant speed during shooting;
3 the warp was always straight and decreased in length at a constant rate during hauling;
4 the net mouth was always perpendicular to the warp;
5 the water velocity varied only with depth (not with longitude, latitude, or time); and
6 the following are known exactly:
   a) the vessel position at the time of shooting and at the start and finish of hauling,
   b) the net depth and warp length at the start of hauling, and
   c) the water velocity profile.

Two further assumptions are described in sections "Calculating P_{n1}" and "Calculating the net path during hauling."

Let $\underline{P}_n(t)$ and $\underline{P}_v(t)$ be 3-dimensional vectors describing the position of the net and the vessel at time t, where time and position are measured in relation to the time and the vessel position when the net was shot, and where the three coordinates give distances, in meters, to the east, north, and downwards, respectively. (In this Appendix, vectors are underlined to distinguished them from scalars.) Let t_1 and t_2 stand for the times at the start and finish of hauling.

Let the water velocity at depth z be described by the vector $\underline{C}(z)$, and the mean water velocity between the surface and depth z by $\underline{C}'(z)$. Denote the net depth and the warp length at time t by $z_n(t)$ and $w(t)$, respectively (note that $z_n(t)$ is the depth coordinate of $\underline{P}_n(t)$).

Where it is convenient, the symbols t_1 and t_2 will be replaced by subscripts 1 and 2, so that, for example, $\underline{P}_n(t_1)$, $z_n(t_1)$, and $\underline{P}_v(t_2)$ may be written as $\underline{P}_{n1}$, z_{n1}, and $\underline{P}_{v2}$, respectively.

From the above assumptions, $\underline{P}_{v1}$, $\underline{P}_{v2}$, z_{n1}, and w_1 are known, as are $\underline{C}(z)$ and $\underline{C}'(z)$ for all z. Also, of course, $\underline{P}_{n2} = \underline{P}_{v2}$.

Calculating $\underline{P}_{n1}$

In calculating the position of the net at the start of hauling, it was assumed that, while the net was sinking prior to hauling, its horizontal velocity was the sum of the water velocity and some fraction c of the vessel velocity (the latter component being caused by drag from the warp). Thus,

$$\underline{P}_{n1} = c\underline{P}_{v1} + t_1\underline{C}'(Z_{n1}) + \underline{Z}_{n1}, \tag{A1}$$

where $0 \le c \le 1$, and $\underline{Z}_{n1}$ is the 3-dimensional vector, $(0,0,z_{n1})$. Also, from assumptions 3 and 6,

$$\left|\underline{P}_{n1} - \underline{P}_{v1}\right| = w_1. \tag{A2}$$

The value of c (and thus $\underline{P}_{n1}$) can be calculated by solving the simultaneous equations A1 and A2. Geometrically, this is equivalent to finding a point of intersection of the horizontal line defined by Equation A1 and the horizontal circle defined by Equation A2 (both are at depth z_{n1}).

Substituting for $\underline{P}_{n1}$ from Equation A1 in Equation A2 and expanding, we get the quadratic equation in (c–1)

$$\begin{aligned}(c-1)^2\left|\underline{P}_{v1}\right|^2 + 2(c-1)t_1\underline{P}_{v1} \bullet \\ \underline{C}'(z_{n1}) + t_1^2\left|\underline{C}'(z_{n1})\right| + z_{n1}^2 - w_1^2 = 0,\end{aligned} \tag{A3}$$

which is solved with the usual formula ($\bullet$ denotes the vector dot product).

Where there were two solutions for c (at all but a few stations), the one using the negative square root was chosen because it was usually the one that satisifed the condition $0 \le c \le 1$.

For the few stations where Equation A3 had no real solution (i.e. the line and circle did not intersect), $\underline{P}_{n1}$ was taken to be the point on the circle closest to the line. It may be shown that this $\underline{P}_{n1}$ is given by

$$\underline{P}_{n1} = r\frac{\underline{P}_{cl}}{\left|\underline{P}_{cl}\right|} + \underline{P}_{v1} + \underline{Z}_{n1} \tag{A4}$$

where r is the radius of the circle and $\underline{P}_{cl}$ is the point, on the surface vertically above the line, that is closest to the shot position, and is given by

$$\underline{P}_{cl} = t_1\underline{C}'(z_{n1}) - \frac{t_1\underline{C}'(z_{n1}) \bullet \underline{P}_{v1}}{\left|\underline{P}_{v1}\right|^2}\underline{P}_{v1}. \tag{A5}$$

Stations where Equation A3 did not have a real solution, or where c did not satisfy $0 \le c \le 1$, were assumed to be instances where the above assumptions did not hold.

Another situation in which the assumptions clearly did not hold was at the 13 stations where $z_{n1} > w_1$. For these stations, z_{n1} was set equal to w_1, and $\underline{P}_{n1}$ was taken to be vertically below $\underline{P}_{v1}$.

Calculating the net path during hauling

From assumptions 1 and 3, the position of the vessel and length of the warp at any time during hauling was calculated by using

$$\underline{P}_v(t) = \frac{(t_2 - t)\underline{P}_{v1} + (t - t_1)\underline{P}_{v2}}{(t_2 - t_1)} \quad \text{(A6)}$$

and

$$w(t) = \underline{P}_v(t) - \underline{P}_n(t) = \frac{(t_2 - t)w_1}{(t_2 - t_1)}. \quad \text{(A7)}$$

To calculate the position of the net during hauling, we made one further assumption: that the velocity of the net is the sum of the water velocity and a vector in the direction of the warp. With this assumption, an iterative procedure was used to calculate $\underline{P}_n(t)$ at times t_1+*t, t_1+2*t, etc, for a small time interval *t. The basis of this procedure is the ability to calculate $\underline{P}_n(t+*t)$ once $\underline{P}_n(t)$ is known. This is done as follows.

The velocity assumption may written approximately as

$$\frac{\underline{P}_n(t+\delta t) - \underline{P}_n(t)}{\delta t} = p(t)\frac{\underline{P}_v(t) - \underline{P}_n(t)}{\left|\underline{P}_v(t) - \underline{P}_n(t)\right|} + \underline{C}\left(z_n(t)\right), \quad \text{(A8)}$$

where $p(t)$ is an unknown scalar which varies with t. Replacing t by t+*t in Equation A7, we get

$$\left|\underline{P}_v(t+\delta t) - \underline{P}_n(t+\delta t)\right| = \frac{(t_2 - t - \delta t)w_1}{(t_2 - t_1)} \quad \text{(A9)}$$

and substituting for $\underline{P}_n(t+*t)$ from Equation A8, Equation A9 may be rewritten as

$$\left|p(t)\underline{A} + \underline{B}\right| = \frac{(t_2 - t - \delta t)w_1}{(t_2 - t_1)}, \quad \text{(A10)}$$

where

$$\underline{A} = -\frac{(t_2 - t_1)\delta t}{w_1(t_2 - t)}\left(\underline{P}_v(t) - \underline{P}_n(t)\right) \quad \text{(A11)}$$

and

$$\underline{B} = \underline{P}_v(t+\delta t) - \underline{P}_n(t) - \delta t\underline{C}\left(z_n(t)\right). \quad \text{(A12)}$$

Expanding Equation A10, we get the quadratic equation

$$\underline{A} \bullet \underline{A}p(t)^2 + 2\underline{A} \bullet \underline{B}p(t) + \underline{B} \bullet \underline{B} = \left[\frac{(t_2 - t - \delta t)w_1}{(t_2 - t_1)}\right]^2, \quad \text{(A13)}$$

which may be solved for $p(t)$ in the usual manner.

In solving Equation A13, the solution using the positive square root was ignored because it led to large values of $p(t)$ and large vertical oscillations in the net path.

Flow of water through the net

Because, by assumption 4 above, the net mouth was always perpendicular to the warp, the flow of water through the net at time t (in m3/s) is given by

$$F(t) = 2\underline{V}_{nw} \bullet \underline{U}_{wp}, \quad \text{(A14)}$$

where V_{nw} is the velocity of the net relative to the water, given approximately by

$$\underline{V}_{nw} = \frac{\underline{P}_n(t+\delta t) - \underline{P}_n(t)}{\delta t} - \underline{C}\left(z_n(t)\right), \quad \text{(A15)}$$

$\underline{U}_{wp}$ is a unit vector in the direction of the warp, given by

$$\underline{U}_{wp} = \frac{\underline{P}_v(t) - \underline{P}_n(t)}{\left|\underline{P}_v(t) - \underline{P}_n(t)\right|}, \quad \text{(A16)}$$

and factor 2 is the net mouth area in m^2.

Calculation of correction factors

The correction factor associated with a horizontal layer of water is given by

$$CF = \frac{\textit{Thickness of layer}}{\textit{Volume of water filtered by net within layer}}. \quad \text{(A17)}$$

Thus, for the layer of water that the net passed through between times t and t+*t, the correction factor is given approximately by

$$\frac{z_n(t) - z_n(t+\delta t)}{F(t)\delta t}.$$

which is treated as the correction factor associated with depth $z_n(t)$. Thus, for each plankton tow, correction factors for depths $z_n(t_1)$ (=z_{n1}), $z_n(t_1$+*$t)$, $z_n(t_1$+2*$t)$, etc. were calculated.

Finally, the correction factor for a given egg stage at a given plankton tow was calculated, by interpolation, as the correction factor at the mid-point of the depth layer associated with that egg stage.

Appendix 2: Calculation of centroids

This appendix describes the procedure for calculating the centroid (= center of gravity) of each age group (plotted in

Fig. 4A). First, the survey area was divided into 36 subareas (Fig. 4A). The longitude of the centroid for age group a, X_a, was estimated by using

$$X_a = \frac{\Sigma_j x_j M_{aj} A_j}{\Sigma_j M_{aj} A_j},$$

where $M_{aj} = \frac{1}{n_j}\Sigma_j C_{aij}$

and C_{aij} = catch rate (eggs/m^2) for age group a at the ith station in subarea j;
M_{aj} = mean catch rate for age group a in subarea j;
n_j = number of stations in subarea j;
A_j = area of subarea j; and
x_j = longitude of center of subarea j.

The latitudes of the centroids were calculated similarly. These calculations use the approximation that, for each age group, the centroid of eggs within a subarea is at the center of the subarea.

Appendix 3: Egg production model and estimation

This appendix describes the calculation of daily egg production, N_0, and CV given in Table 4 and shown in Figure 6. It is assumed that

1 the rate of egg production was constant (both from day to day and within each day) in the period immediately preceding and during the plankton survey,
2 egg mortality was constant over the same period and independent of age, and
3 the egg abundance estimates, N_a, of Table 3 are unbiased and lognormally distributed with CV's, c_a, as given in Table 3.

Under these assumptions, E_a, the expected value of N_a, is given by

$$E_a = \int_{t_{a-1}}^{t_a} N_0 e^{-zt} dt = \frac{N_0}{Z}\left(e^{-Zt_{a-1}} - e^{-Zt_a}\right),$$

where N_0 = the daily egg production (eggs/day);
Z = the daily instantaneous egg mortality (per day); and
(t_{a-1}, t_a) = the range of ages (day) in age group a.

The mean age of eggs of age group a (used in Fig. 6) is given by

$$\frac{1}{E_a}\int_{t_{a-1}}^{t_a} tN_0 e^{-Zt} dt = \frac{1}{Z} + \frac{\left(t_a e^{-Zt_a} - t_{a-1} e^{-Zt_{a-1}}\right)}{e^{-Zt_a} - e^{-Zt_{a-1}}}.$$

N_0 and Z were estimated by maximum likelihood, ie. by maximizing the likelihood, L, which is given by (ignoring constants)

$$L = \prod_a \left[\frac{1}{N_a}\exp\left(-0.5\sum_a\left[\frac{1}{\sigma_a}\log\left(\frac{N_a}{E_a}\right) + 0.5\sigma_a\right]^2\right)\right],$$

where σ_a is the standard error of log (N_a), given by

$$\sigma_a = \left[\log(1+c_a^2)\right]^{\frac{1}{2}}.$$

By definition, Z must be positive. However, in the bootstrap procedure described next, it sometimes happened that the maximum likelihood estimate of Z was negative (because, by chance, simulated egg abundance estimates increased with age). When this happened, Z was forced to be zero, so that

$$E_a = N_0(t_a - t_{a-1}).$$

The following bootstrap procedure was used to estimate the degree of uncertainty in the estimates of N_0 and Z.

1 the maximum likelihood estimates of N_0 and Z were used to calculate the expected egg abundances, E_a, using the formula above;
2 new egg abundance estimates, N_a, were simulated by using lognormal distributions with expected values, E_a, and CV's, c_a;
3 maximum likelihood estimates of N_0 and Z were calculated from the simulated values of N_a, and
4 steps 2 and 3 were repeated 1,000 times.

The resulting 1,000 values of N_0 and Z are the bootstrap distributions for these parameters; the 0.025 and 0.975 quantiles of these distributions were taken as bounding the 95% confidence intervals for each parameter. The CV's of these distributions are taken as estimates of the CV's of the maximum likelihood estimates of N_0 and Z.

Appendix 4: Analysis of ovarian samples

Ovaries frozen onboard were thawed in the laboratory, weighed, and then one of the two slit open. A subsample of about 5 g was scraped from the full length of one ovary from each fish, because preliminary analyses had indicated there was some variation in egg density from different parts of the ovary. The subsample was then placed into 1M KOH for a period of 5–15 minutes depending on the ovarian stage. Maturing oocytes (stage 3) were bound more tightly in the matrix than advanced stages, and therefore needed a longer KOH treatment to separate individual oocytes. Stage-3 oocytes generally retained a medium orange color after KOH treatment, and thus needed no further treatment after separation. However, the more transparent stage-4, stage-5, and stage-8 oocytes were stained with Semichon's carmine. The subsample was then washed through a 0.7-mm mesh sieve that was found to be of a suitable mesh size to retain oocytes with a diameter greater than about 1.2 mm. Primary, early vitellogenic, and atretic oocytes, as well as small fragments of matrix and tissue

passed through the mesh. Atretic eggs, in particular, had dimensions of 0.5 to 1.0 mm (from histological observations) and were found to wash through the sieve. The mean size of stage-3 oocytes was 1.56 mm (range 1.30–1.83 mm, SE=0.01). More advanced stages had larger oocytes (stage 4=2.15 mm; stage 5= 2.55 mm; stage 8=2.20 mm) and therefore were fully retained.

Eggs were then counted with an electronic egg counter incorporating a phototransistor (Bycroft, 1986). Eggs were siphoned from a large beaker into a perspex chamber with a vacuum pump attached to the top of the chamber. Water flow was regulated to keep this chamber about three-quarters full, so that any air bubbles rose to the top. Eggs passed from the bottom of the chamber through a small tube past a phototransistor and light source. The reduction in light intensity (caused by the presence of the eggs) was detected by the phototransistor and was recorded on an electronic counter with digital readout. The rate of flow was regulated to avoid large numbers of eggs in the chamber and exit tube at any one time (which could result in multiple eggs passing the counter at the same time and being recorded as only as one egg). A gain control (set very low) was used to alter the sensitivity of the counter so that small fragments of extraneous material that remained after the sieving were not counted.

Checks of the accuracy of the electronic counter were made by comparing manual counts (made with a dissecting microscope) with electronic counts and no significant difference was found (t-test, $P<0.01$, number of comparisons=4). Also, a reference set of a known number of eggs was regularly used to check the calibration. Up to four determinations were made in each trial, which produced CV's of the mean count of generally less than 0.01.

Total fecundity from both ovaries was initially calculated from the number of oocytes/g × ovarian weight. Although this is common practice in fecundity studies, it may introduce bias because ovary weight includes ovary wall which does not contain eggs. Several comparisons were made between estimated fecundity based on the scaled-up subsample count, and actual counts of all oocytes in an ovary. Scaled-up subsample counts typically overestimated actual fecundity by 10–20%. The proportion of wall weight to total ovary weight (for stages 3, 4, 5, and 8) were estimated by stripping a number of weighed ovaries of all eggs and weighing the remaining wall (Appendix 4, Table 1). The proportions were then used to adjust the total fecundities of each fish in each stage. The higher proportions for stages 5 and 8 are to be expected because some stage-5 fish may be in the act of spawning and stage-8 fish have already spawned some of their eggs; therefore the ovary wall is a greater proportion of total ovary weight.

Table 1

The contribution (percentage) of the ovary wall to ovary weight at each ovarian stage. See text for stage definitions.

Stage	Mean	SE	n
3	9.6	1.2	15
4	6.6	0.4	14
5	13.9	2.7	14
8	29.7	4.4	19

Maturity and fecundity of arrowtooth flounder, *Atheresthes stomias*, from the Gulf of Alaska

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Abstract.—Maturity and total fecundity are reported for arrowtooth flounder, *Atheresthes stomias*, from the Gulf of Alaska. Histological examination of both ovarian and testicular tissues revealed that the maturity state of both sexes could not be determined reliably by macroscopic assessment. Maturity for females ranged from the early perinucleus to the migratory nucleus stage; none of the fish had postovulatory follicles or hydrated oocytes, indicating all samples were collected prior to the spawning season. Condition factor (CF), gonadosomatic index (GSI), and hepatosomatic index (HSI) increased significantly in the later stages of female development. Eyed-side ovarian lobes were significantly heavier than blind-side lobes, but oocyte size and density (oocytes/gram) did not vary between lobes of the ovary or within the individual ovarian lobes. Total fecundity increased exponentially with length ($F=0.0429 \times L^{4.020}$) and linearly with somatic weight ($F=350.4 \times W - 138{,}482$), with estimates ranging from 250,000 to 2,340,000 oocytes. Histological analysis of tissues indicated that females reach 50% maturity (L_{50}) at 47 cm, males at 42 cm. This estimate of male L_{50} is probably high because no males in this study were ready to spawn, whereas a decrease in CF and an increase in GSI indicate body changes at a size of 30–35 cm.

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Arrowtooth flounder, *Atheresthes stomias*, is a large piscivorous flatfish with a range in U.S. waters from California (Allen and Smith, 1988) through the Gulf of Alaska, the Aleutian Islands, and the eastern Bering Sea. Although it is the most abundant groundfish in the Gulf of Alaska and is concentrated in relatively shallow waters (101–200 m, Martin and Clausen, 1995), arrowtooth flounder has experienced only limited commercial harvesting. Arrowtooth flounder flesh softens soon after capture, possibly owing to an enzyme released from a myxosporean parasite (Greene and Babbitt, 1990), greatly reducing its commercial value. Recent advances in food processing, however, have allowed production of marketable quality arrowtooth flounder fillets (Greene and Babbitt, 1990) and surimi (Wasson et al., 1992; Porter et al., 1993; Reppond et al., 1993), which may stimulate further development of a fishery.

Hunter and Macewicz (1985, a and b), Hunter et al. (1992), Morrison (1990), and others have carefully examined many of the assumptions of maturity and fecundity work and have provided histological details that made this project possible. Rickey's (1995) research off the Washington coast has shown that arrowtooth flounders are group-synchronous batch spawners, and researchers generally agree on a fall or winter spawning period (Pertseva-Ostroumova, 1961; Shuntov, 1970; Fargo et al., 1981; Rickey, 1995; Hosie and Barss[1]). Studies on arrowtooth flounder have calculated length at 50% maturity (L_{50}) for both males and females from different areas, but only by using macroscopic maturity assessment (Hosie and Barss[1] for Oregon; Fargo et al., 1981, for British Columbia, Canada; Rickey, 1995, for Washington). Rickey (1995) included histological analysis and descriptions of different maturity stages of ovarian tissues but did not use histological analysis for calculating L_{50} and did not examine males histologically.

No study has ever reported weight-based or length-based estimates of total fecundity (as defined in Hunter et al., 1992), nor have researchers examined possible differences in oocyte density or oocyte size between or within ovarian lobes, which could bias total fecundity estimation. Researchers also have not reported changes in condition factor, gonadosomatic index, and hepatosomatic index with maturity stage; such information could provide useful information on development.

In this study, I report on the maturity and total fecundity of arrowtooth flounder in the Gulf of Alaska.

[1] Hosie, M. J., and W. H. Barss. 1977. Age and length at maturity of arrowtooth flounder, *Atheresthes stomias*, in Oregon waters. Marine Field Laboratory, Oregon Dep. Fish and Wildlife, P.O. Box 5430, Charleston, OR 97420. Unpubl. manuscr., 9 p.

Histology is used to determine maturity, and detailed descriptions of maturity stages (adapted from Rickey, 1995) are provided for both males and females. Changes in condition factor, gonadosomatic index, and hepatosomatic index are reported for the different maturity stages. Another objective of this study is to assess methods for describing arrowtooth flounder maturity (macroscopic vs. histologic) and for determining total fecundity. The accuracy of macroscopic maturity staging is assessed by comparing histological staging with macroscopic staging for all fish sampled, since Rickey (1995) noted the possibility of misassigning maturity stages by macroscopic means. Comparisons are made between this study and results from arrowtooth flounder maturity studies conducted from the eastern Bering Sea to Oregon.

Materials and methods

Maturity

Arrowtooth flounder were collected from bottom trawl hauls aboard the NOAA vessel *Miller Freeman* (cruise 93-10) on Portlock Bank near the eastern end of Kodiak Island, Alaska. The hauls were made during daylight hours from 6 to 16 September 1993 in depths ranging from 66 to 165 m. Fork lengths (cm) were recorded, and somatic fish weights (less stomach contents) were measured to ±2 g on an electronic scale. The gonadal tissues were removed from the fish within a few hours of capture and preserved in a 10% formalin solution neutrally buffered with sodium acetate. Eyed-side and blind-side lobes of the ovary were dissected apart and stored separately. Livers were removed and weighed on the vessel (±2 gm). Specimens less than 20 cm in length were weighed on the vessel and preserved whole in a 10% formalin solution. Preserved fish were later dissected in the laboratory, and liver weights were measured to ±0.001 gm.

The author assigned a macroscopic maturity stage to males and females (Table 1) based on external appearance of gonads, according to descriptions adapted from Rickey (1995). (The author is an experienced groundfish biologist and prior to this project had examined the appearance of arrowtooth flounder gonadal tissue, through sex determination work, on thousands of arrowtooth flounders). Collections were initially limited to two fish per centimeter for each sex; additional females were collected over the length range where macroscopic maturity stages overlapped.

The accuracy of macroscopic stages assigned to whole ovaries was assessed by histological analysis of the ovarian sections. After being preserved in formalin, gonadal tissues were blotted dry and weighed on an electronic scale (±0.001 g) in the laboratory. Sections for histology were approximately 3 mm thick. For females, the sections were cut perpendicularly through the eyed-side ovarian lobe as near to the posterior end of the lobe in order for the section to fit on a slide. For males, the section was cut perpendicularly through a distal lobe of the testis, or through the entire organ, if small. Histological samples were dehydrated, infiltrated with paraffin, and embedded in blocks of paraffin. Sections were cut from the frozen blocks on a microtome at a thickness of 5 μ, heat-fixed to a glass slide, and stained with hematoxylin and eosin. Under a compound microscope, the ovary samples were assigned one of 11 maturity stages on the basis of the most advanced oocyte seen (Table 2). Atresia of large, yolked oocytes was noted, but not quantified.

The gonadosomatic index (GSI) was calculated to show differences in development of the gonads with respect to body weight:

$$GSI = (\textit{gonad weight} \times 100)/\textit{somatic weight}.$$

Condition factor (CF) was calculated as an overall measure of robustness of the fish:

$$CF = (\textit{somatic weight} \times 100)/\textit{length}^3.$$

A hepatosomatic index (HSI) was also calculated to estimate the relative size of the liver to body weight:

$$HSI = (\textit{liver weight} \times 100)/(\textit{liver} - \textit{free somatic weight}).$$

Although HSI is generally not included in maturity studies, the liver plays an important role in sexual maturity of both sexes. Oocyte yolk comes from the manufacture of vitellogenin in the liver (Wallace, 1985), and HSI may be a good predictor of male gonadal development, as was shown for Pacific cod (*Gadus macrocephalus*; Smith et al., 1990).

Significant differences between mean values of length, weight, GSI, CF, and HSI at the different maturity stages were tested with a one-way analysis of variance (ANOVA, α=0.05). The mean values were further tested with a Tukey test to reveal which means were significantly different. For purposes of these tests, fish in the late perinucleus stage (stage 4) were combined with those that had atresia of previously vitellogenic oocytes but that did not yet have mature oocytes beyond the late perinucleus stage (stage 11) in the current season.

Females were classified as mature if their oocytes had entered the cortical alveoli stage (stage 5; Rickey,

1995) or showed atresia of vitellogenic oocytes. Males whose testes contained either spermatids or spermatogonia were classified as mature. The proportion mature at each length was calculated by

$$P_x = 1/(1+e^{ax+b}),$$

where P_x is the proportion mature at a given length x, and a and b are constants. Size at fifty percent

Table 1

Macroscopic and histological descriptions of developmental stages used to classify male and female arrowtooth flounder, *Atheresthes stomias* (adapted from Rickey, 1995). Mean oocyte diameter in parentheses.

Macroscopic stage and description	Histological stage and description
Females	
A Immature Ovaries small and pink with no oocytes visible.	**1 Oogonia** Very small (2.5 μ) and staining dark purple.
	2 Chromatin nucleolus Nucleus large, one nucleolus, cytoplasm layer thin, both staining dark purple (25–75 μ).
	3 Early perinucleus Nuclear material granular, several nucleoli around perimeter of dark-purple-staining nucleus, lighter purple vacuoles (cortical alveoli) forming around nucleus and moving outward in dark-purple-staining cytoplasm. Cytoplasm growing in thickness (37.5–75 μ).
	4 Late perinucleus Material in nucleus often moving to one side leaving much of nucleus clear, becoming fibrous with lampbrush chromosomes. Many nucleoli evenly spaced around perimeter of nucleus, cytoplasm staining less purple, ring of light purple vacuoles (cortical alveoli) still moving outward, sometimes dividing cytoplasm into two zones (150–162.5 μ).
B Developing Ovaries white to yellow, firm, oocytes visible.	**5 Cortical alveoli** Some lampbrush chromosomes still present, but nuclear material dispersing and nucleus turning light purple, collapsing inward. Vacuoles in cytoplasm (cortical alveoli) near cell wall, clear, one to three layers thick, cytoplasm much lighter purple (325–375 μ).
	6 Early vitellogenesis Nucleus light purple to pink in color, collapsing inward. Cortical alveoli increasing in diameter, on the outer margin of the cell wall. First yolk globules (pink) in cytoplasm, generally closer to the center of the oocyte than the cortical alveoli, sometimes in spokelike configuration. Cytoplasm less than 50% filled with yolk (375–425 μ). Yolk globules 7.5 μ.
	7 Late vitellogenesis Pink-staining yolk globules occupy 50–100% of the cytoplasm. Oocyte diameter 500 μ and yolk globules expanding to 12.5 μ.
	8 Migratory nucleus Yolk globules coalescing and increasing in diameter (25–37.5 μ), nucleus sometimes visible as a crescent shape, cortical alveoli no longer visible near edge of cytoplasm, oocyte large (625–725 μ).
C Gravid Hydrated oocytes present.	**9 Hydrated** Nucleus no longer visible, yolk coalescing and filling cytoplasm as continuous material.
D Ripe and running Oocytes extruded with light pressure.	**10 Spawning** Postovulatory follicles present.
E Spent or resting Ovaries bloodshot, flaccid, and dark red to purple.	**11 Atretic** Atresia of large, previously yolked oocytes, no stages beyond late perinucleus present.
Males	
Immature Testes small and threadlike, pink.	Spermatogonia, primary or secondary. Spermatocytes present.
Mature Testes enlarged, folded, brown or white in color.	Spermatids or spermatogonia present.

maturity was estimated by substituting 0.5 for P_x. The constants a and b were estimated through iterative, nonlinear regression by the StatGraphics Plus (version 6.1 for DOS) program.

Total fecundity

Weighed samples (±0.0001 g) of whole oocytes from the most mature females (stage 8, Table 1) were counted and measured under a dissecting scope with Optimas image analysis software, and numbers were expanded to the whole ovary by using the gravimetric method. Only yolked oocytes, which were opaque, appeared as dark images on the computer monitor with back lighting. Immature oocytes, which were without yolk, were translucent and not discernible on the screen. The Optimas software automatically measured the area of the oocytes by delimiting the circumference, from which the diameters were derived. Individual measurements of oocyte diameters were normalized with a cubic transform (diameter^3) because the distributions were skewed to the left (negatively) (Zar, 1984). Possible differences of oocyte density (oocytes/gram) and mean oocyte diameters between ovary lobes were tested with paired t-tests. Two-way ANOVA's were used in testing for differences in oocyte density and diameter among anterior, medial, and posterior positions within the same ovarian lobe.

Possible differences between the weights of the eyed-side and blind-side ovarian lobes were tested with a paired t-test, and the relation between the lobe weights was described with a linear regression.

Results

Maturity

Gonadal tissue samples were collected from a total of 176 female and 58 male arrowtooth flounder. Damage to the ovarian lobes, or other tissues, and loss of ovarian tissue during dissection and storage were common in the larger, more mature females. This damage or loss of tissue resulted in a lower sample size (n=158) for measurements such as GSI and HSI. In addition, the ovarian lobes of three fish were so small that the lobes were not separated during dis-

Table 2

Histological analysis of macroscopic maturity stages.

Macroscopic maturity stage		Microscopic maturity stage	Count	Percent	Number with atresia	Percent per stage with atresia
Stage A (Immature)		3	11	14.5	0	0.0
		4	51	67.1	0	0.0
		5	6	7.9	5	83.3
		11	8	10.5	8	100.0
	Subtotal		76		13	17.1
Stage B (Developing)		5	1	2.0	1	100.0
		6	3	6.0	1	33.3
		7	31	62.0	6	19.4
		8	15	30.0	0	0.0
	Subtotal		50		8	16.0
Stage E (Spent or resting)		4	2	4.4	0	0.0
		5	13	28.9	6	46.2
		6	11	24.4	2	18.2
		7	11	24.4	1	9.1
		8	4	8.9	0	0.0
		11	4	8.9	4	100.0
	Subtotal		45		13	28.9
Stage A or E (Developing, or spent or resting)		7	3	100.0	1	33.3
Unidentified		4	1	50.0	0	0.0
		6	1	50.0	0	0.0
	Total		176			

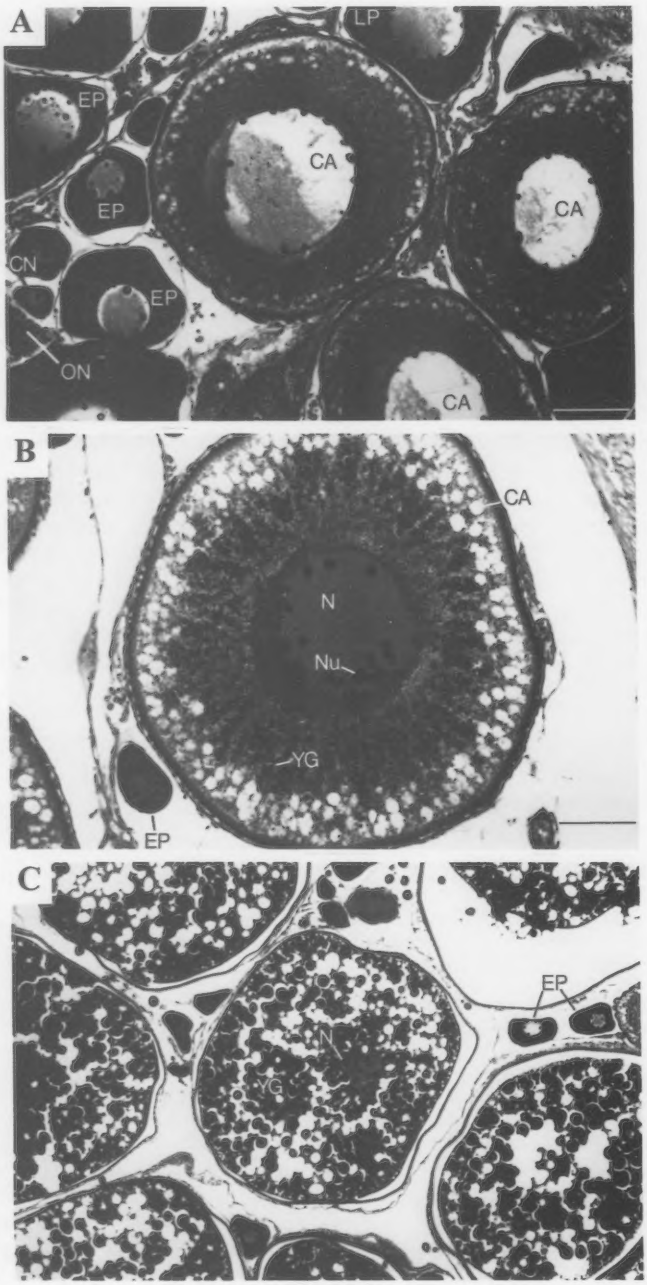

Figure 1

Oocytes in arrowtooth flounder, *Atheresthes stomias*, at different stages of development. (**A**) Oocytes of a 57-cm-FL female at the cortical alveoli stage as well as less mature oocytes. (**B**) Oocyte of a 66-cm-FL female at the early vitellogenesis stage. (**C**) Oocytes of a 82- cm- FL female at the migratory nucleus stage. Bar = 0.1 mm; ON = oogonial nest; CN = chromatin nucleolus; EP = early perinucleus; LP = late perinucleus; CA = either cortical alveoli stage oocytes or cortical alveoli structures (with arrow); N = nucleus; Nu = nucleolus; YG = yolk globule.

section, making them unavailable for the lobe-weight comparison.

In the five-tier macroscopic staging scale for females (Table 1), fish were classified only as "immature" (stage A), "developing" (stage B), or "spent or resting" (stage E). No females were found with hydrated oocytes ("gravid," stage C) or that were "ripe and running" (stage D). The two-tier macroscopic maturity scale for males was abandoned early in the study because of lack of confidence in assigning stages—nearly all males appeared to be "immature."

Histological analysis revealed that all females were in stages 3–8 and stage 11 of oocyte development (from early perinucleus to the migratory nucleus stage, and the atretic stage, Table 2). The lack of hydrated oocytes or postovulatory follicles in any of the ovaries indicated that these samples were collected prior to the spawning season. None of the males were ready to spawn but in some specimens, spermatids and spermatozoa were present, indicating that the males were preparing to spawn.

Figure 1 shows photographs of histological sections taken from females at three different stages of maturity. The progression of size increase of oocytes from the oogonial nest stage through the cortical alveoli stage can be seen in Figure 1A. Some specimens in early vitellogenesis, such as shown in Figure 1B, had yolk globules arranged in a spokelike configuration in the cytoplasm. The increase in oocyte size in the migratory nucleus stage, in comparison with oocyte size in the early perinucleus stage, and the increase in size of yolk globules are shown in Figure 1C.

The categories of maturity stages, based on macroscopic examination, are shown in Table 2. Most of the females classified macroscopically as "immature" (stage A, n=76, length range 14–64 cm) were in the early or late perinucleus stage (n=62, 81.6%). A total of 18.4% (14 of 76) of these females classified macroscopically as "immature" were either in the process of maturing (cortical alveoli stage) or showed evidence that they had been mature the previous season (atresia of previously yolked oocytes).

Table 3

Tukey test results showing significant differences (<) and equalities (___) between arrowtooth flounder (*Atheresthes stomias*) females in different stages of development, as determined by histology (EP = early perinucleus, LP = late perinucleus, CA = cortical alveoli, EV = early vitellogenesis, LV = late vitellogenesis, and MN = migratory nucleus).

Variable	Histological stage											
Length	EP	<	LP	<	CA		EV		LV			MN
Weight	EP		LP	<	CA		EV		LV			MN
Condition factor	EP		EV		CA		LP	<	LV	<		MN
Gonadosomatic index	EP		LP	<	CA	<	EV	<	LV	<		MN
Hepatosomatic index	EP		LP		CA		EV	<	LV	<		MN

Histological examination of females classified macroscopically as "developing" (stage B, n=50, length range 47– 83 cm) revealed that they were in vitellogenesis and thus correctly classified. Most of the females had oocytes that were in some phase of yolk acquisition (early vitellogenesis to migratory nucleus stages), and only 2.0% were in the cortical alveoli stage. Sixteen percent of these fish had atretic oocytes.

Most of the females categorized as "spent or resting" (stage E, n=45, length range 49–83 cm) were in some stage of vitellogenesis (n=39, 86.7%) and should have been classified as "developing." The percentage of fish with atresia declined with increasing maturity stage, from 46.2% in the cortical alveoli stage to 0.0% in the migratory nucleus stage. Overall, 28.9% of these "spent or resting" fish had atresia, the highest rate of all three macroscopic stages. Only 4 fish out of 45 were correctly determined to be "spent or resting" (late perinucleus with atresia).

Three females were assigned a combined macroscopic classification of "developing" or "spent or resting." In two of the fish the anterior portion of the ovarian lobes appeared to be "developing" whereas the posterior ends appeared to be "spent or resting." In the third fish the blind-side ovarian lobe appeared to be "developing" whereas the eyed-side appeared to be "spent or resting." Histological examination demonstrated that all three of these fish were in late vitellogenesis; even samples taken from the portions of the ovary that were macroscopically classified as "spent or resting" demonstrated that the fish were in late vitellogenesis.

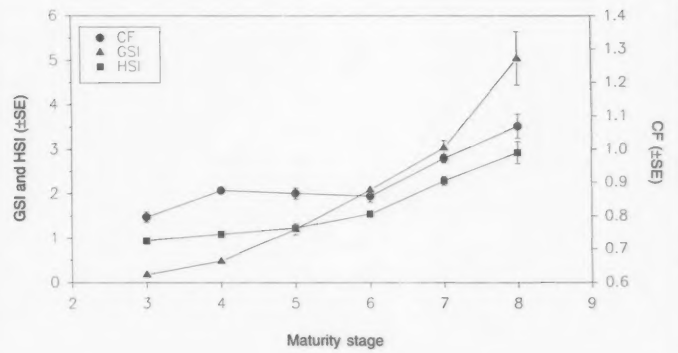

Figure 2

Mean values (±SE) of condition factor (CF), gonadosomatic index (GSI), and hepatosomatic index (HSI) for the stages of female arrowtooth flounder maturity, as determined by histology.

I failed to classify two females macroscopically because of size-based bias. A large fish (66 cm), in which the ovary appeared to be "immature," was actually in the early vitellogenesis stage. A small fish (20 cm), in which the ovary appeared to be "developing," was in the late perinucleus stage.

Mean values of CF, HSI, and GSI of the histological stages for females are presented in Figure 2, with significant differences shown in Table 3. Condition factors of the first four stages were not significantly different, but CF was higher in the late vitellogenesis stage and highest in the migratory nucleus stage (Single-factor ANOVA; df=5, F=25.9, P<0.001; Tukey tests). The small decline in mean CF values between

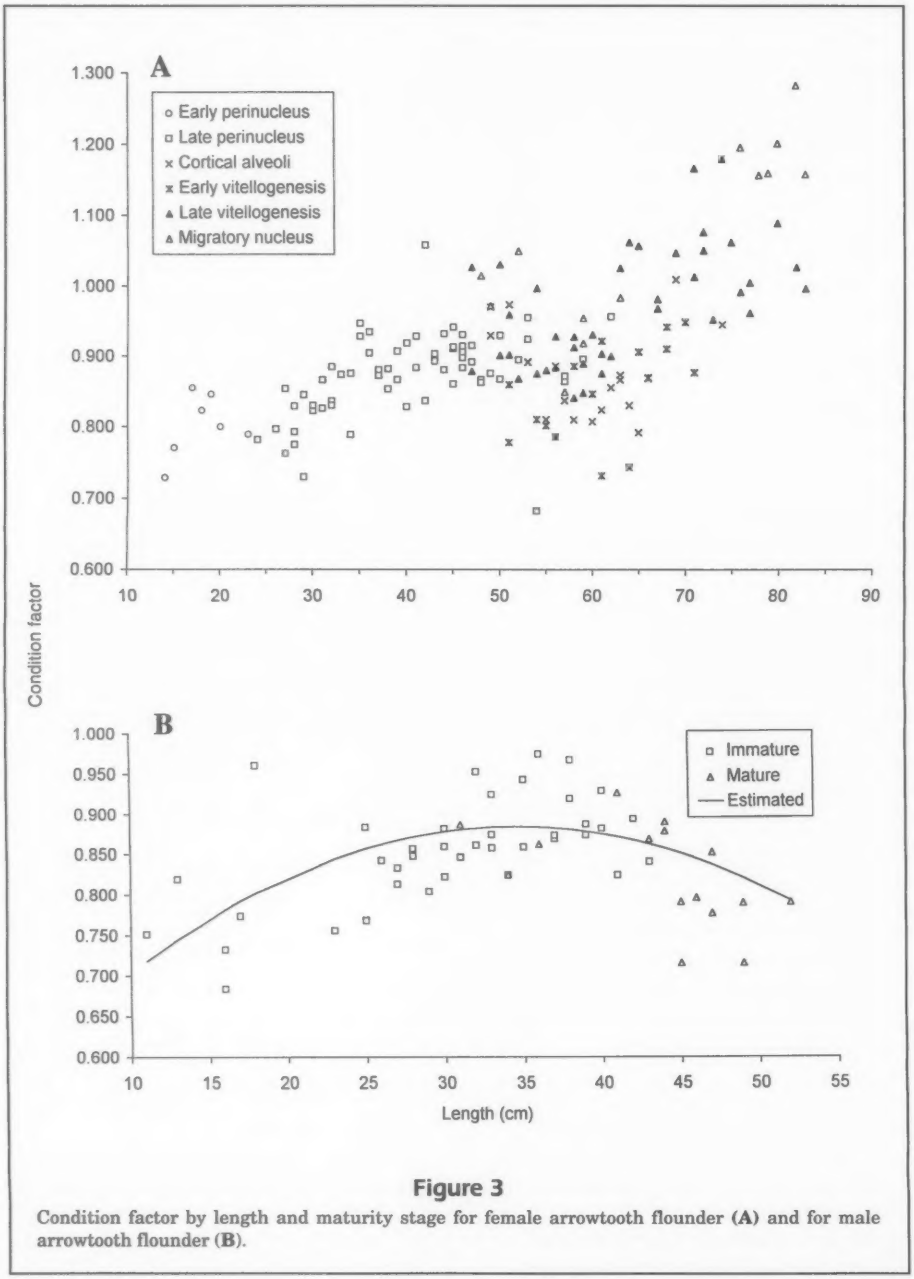

Figure 3

Condition factor by length and maturity stage for female arrowtooth flounder **(A)** and for male arrowtooth flounder **(B)**.

stages 4, 5, and 6 was not significant (Fig. 2), but there was a similar decline in CF in fish between 50 and 60 cm in length (Fig. 3A). Condition factor of immature males (0.854) was not significantly different (t-test; df=56, $P>0.05$) from that for mature males (0.822); however, the regression fit of the relationship (df=55, $F=4680$, $P=<0.001$, $r^2=0.31$) shows that CF increased with length for small fish, reached a peak at 34.5 cm, and declined in larger fish (Fig. 3B).

Hepatosomatic index varied significantly among female maturity groups (single-factor ANOVA; df=5, $F=59.0$, $P<0.001$) and was significantly greater in both the late vitellogenesis stage (2.281) and migratory nucleus stage (2.921). In general, HSI increased

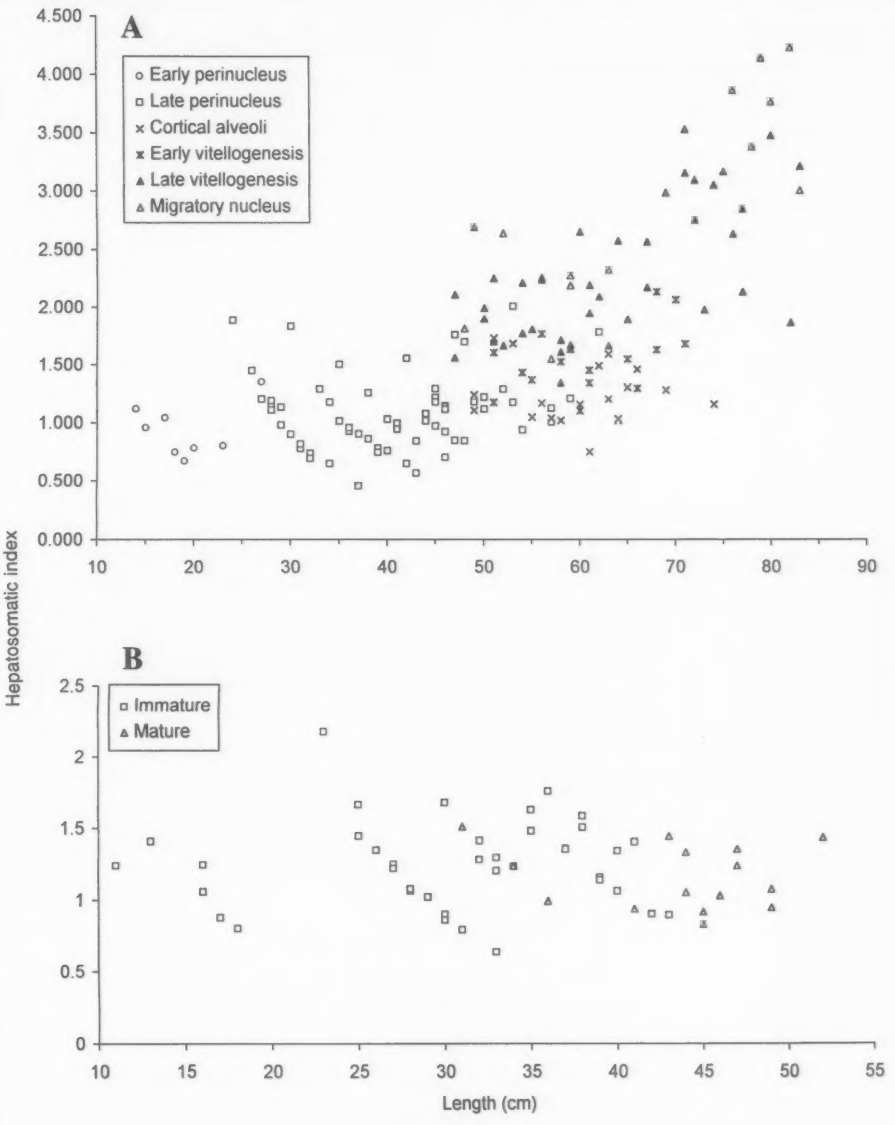

Figure 4

Hepatosomatic index by length and maturity stage for female arrowtooth flounder (**A**) and for male arrowtooth flounder (**B**).

with increasing female length (Fig. 4A). There was no difference in HSI between mature and immature males (unpaired *t*-test; df=27, $P>0.05$), and no trends in the data (Fig. 4B).

Gonadosomatic index varied significantly among female maturity groups (single-factor ANOVA; df=5, $F=91.9$, $P<0.001$). The Tukey test revealed that despite more than doubling, the GSI did not change significantly between the early and late perinucleus stages (Table 3). After that, the GSI was significantly greater in each succeeding stage of maturity (Table 3). Figure 5A shows that GSI remained low until fish reached lengths over 45 cm. GSI was significantly greater in mature males than in immature males

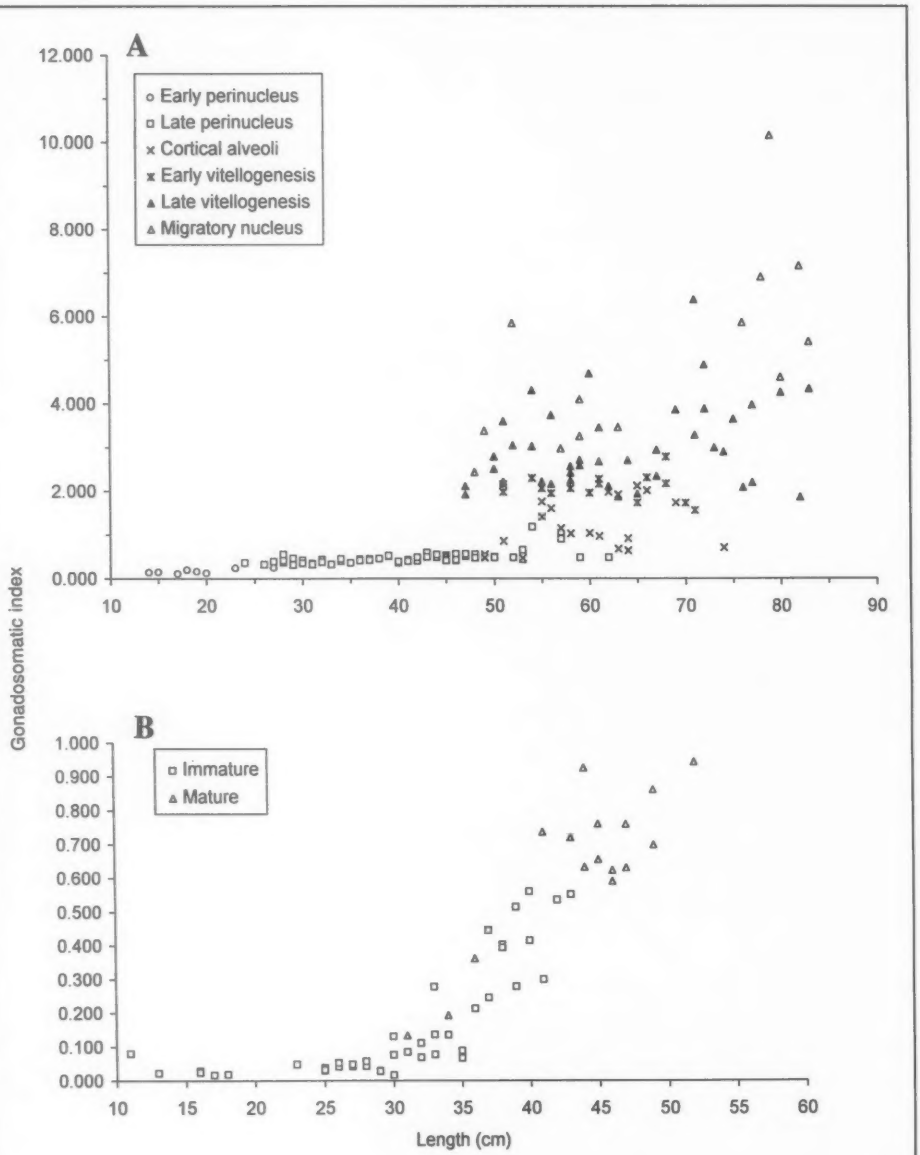

Figure 5

Gonadosomatic index by length and maturity stage for female arrowtooth flounder (**A**) and for male arrowtooth flounder (**B**).

(unpaired t-test; df=27, $P<0.001$); the increase began between 30 and 35 cm in length (Fig. 5B).

According to histological analysis, L_{50} for females occurred at 46.9 cm (df=174, F=897, r^2=0.76, Fig. 6A) and at 42.2 cm (df=56, F=69, r^2=0.60, Fig. 6B) for males. According to macroscopic staging, L_{50} for females occurred at 49.9 cm (df=172, F=748, r^2=0.76, Fig. 6A).

Total fecundity

In general, the eyed-side ovary lobes were heavier than the blind-side lobes (paired t-test; df=154, t=2.663, P=0.009). Linear regression analysis (df=153, F=2365, $P<0.001$, r^2=0.94) described the relation between the weight of ovarian lobes as

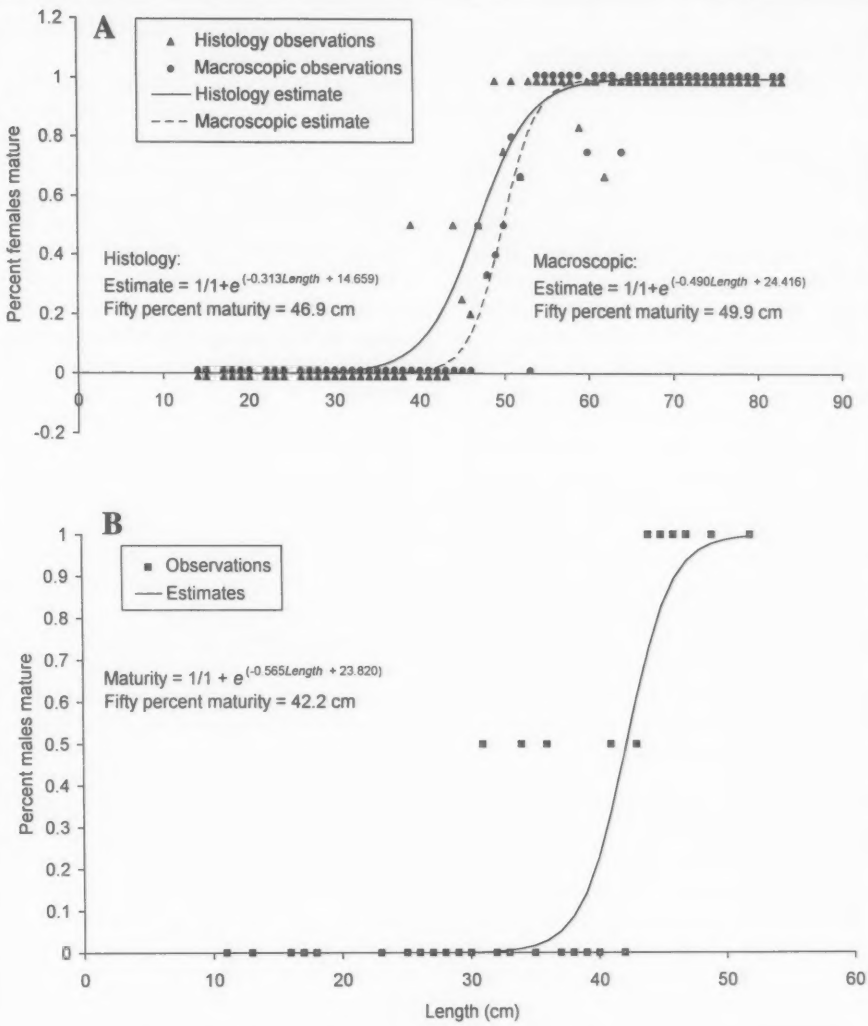

Figure 6

Percent maturity by length for female arrowtooth flounder, as determined from histology and macroscopic observations (**A**); percent maturity by length for male arrowtooth flounder (**B**).

$$W_B = 0.843(W_E) + 1.398,$$

where W_B = the weight of the blind-side lobe; and
W_E = the weight of the eyed-side ovary lobe.

All the frequency distributions of the diameter of maturing oocytes were unimodal and had a long tail on the left. A cubic transform (diameter^3) normalized the distributions. The transformed values of the mean diameter of oocytes did not vary significantly among positions (anterior, medial, posterior) within the same ovarian lobe (two-factor ANOVA, df=2, $P>0.05$) or between lobes (paired t-test, df=9, $P>0.05$). Density of oocytes (count of oocytes per gram of ovarian tissue) also did not vary significantly between positions within ovarian lobes (two-factor ANOVA, df=2, $P>0.05$) or among ovarian lobes (paired t-test, df=9, $P>0.05$). Thus samples for total fecundity were combined from the different positions and ovarian lobes.

Total fecundity was significantly related to fish length (df=11, $F=125.7$, $P<0.001$, $r^2=0.92$) by the equation

$$\ln(F) = 4.020 \ln(L) - 3.149,$$

from which was derived

$$F = 0.0429 (L)^{4.020},$$

where F = total fecundity; and
L = fork length in centimeters (Fig. 7A).

Length-based total fecundity estimates ranged from 246,000 (48 cm) to 2,224,000 (83 cm) oocytes. Total fecundity was also significantly related to somatic fish weight (df=11, F=264.7, P<0.001, r^2=0.96):

$$F = 350.4(W) - 138{,}482,$$

where F = total fecundity; and
W = somatic weight in grams (Fig. 7B).

Weight-based total fecundity estimates ranged from 255,000 (1,122 g) to 2,339,000 (7,070 g) oocytes.

Discussion

Maturity

Histological analyses demonstrated that the assignment of macroscopic maturity stages was not always reliable. Although it was confirmed through histol-

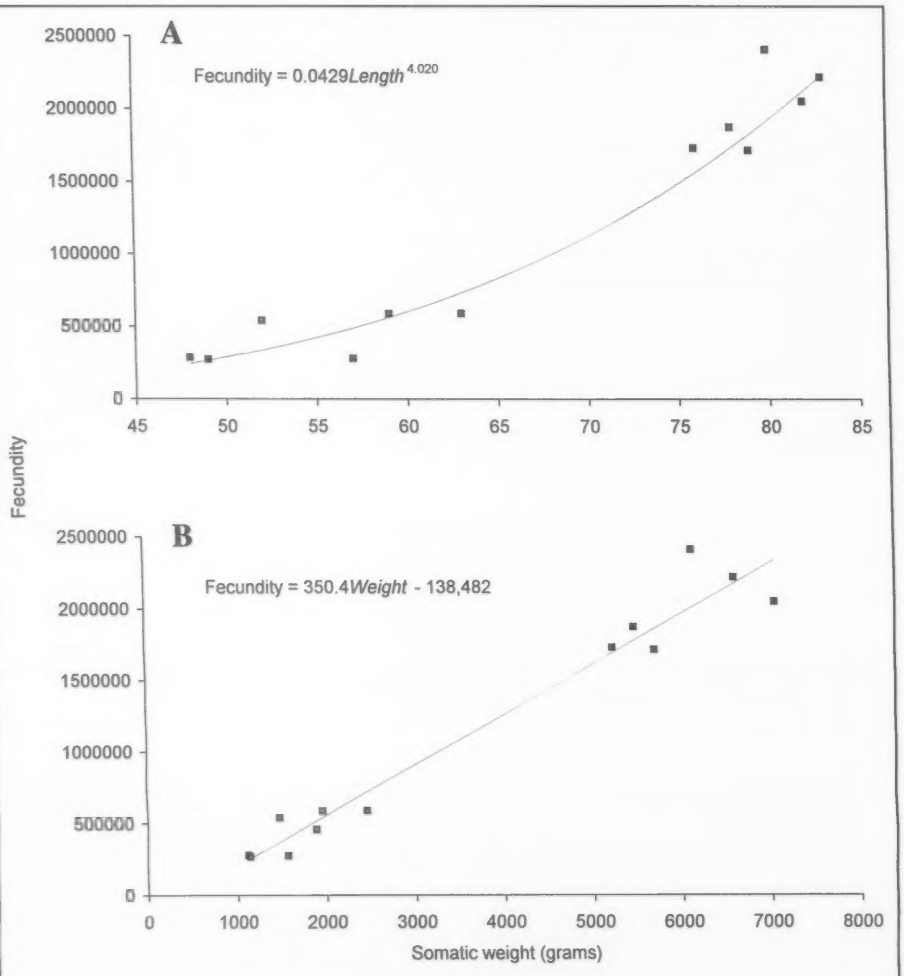

Figure 7

Total fecundity by length for female arrowtooth flounder (**A**); total fecundity by weight for female arrowtooth flounder (**B**).

ogy that none of the "immature" females had begun acquiring yolk, some had begun the process of maturing oocytes, and several had atresia of large, previously yolked oocytes, indicating that these females had probably spawned during the previous year. All females classified macroscopically as "developing" had maturing oocytes to spawn, most of them in the later stages of vitellogenesis. Few of the fish classified as "spent or resting" were actually resting: most were in vitellogenesis and should have been classified as "developing." In addition, the author was unable to assign a single maturity stage to ovaries with a mixed appearance and to assign a stage for ovaries from some females.

The several females correctly classified as "spent or resting" deserve some discussion. These fish had oocytes only as advanced as the late perinucleus stage and had degenerating oocytes present, which had previously been yolked. These fish were classified as mature because they had previously contained yolked oocytes, but they were unable to spawn soon, despite the upcoming spawning season, and they did not show signs of recent spawning. Hunter and Macewicz (1985, a and b) have reported histological details on creation and resorption of atretic oocytes and postovulatory follicles in the northern anchovy, *Engraulis mordax*. Their results have shown that the relatively rapid resorption of yolked oocytes (after 23 days of starvation; Hunter and Macewicz, 1985a) and postovulatory follicles (after 3–4 days; Hunter and Macewicz, 1985b) seems to contradict my assertion that atretic vitellogenic oocytes from the previous spawning season were still being resorbed in arrowtooth flounder just prior to the spawning season. A comparison of the reproductive cycle of arrowtooth flounder (which is a determinate-spawning benthic flatfish, dwelling in relatively cold northern waters) with that of the northern anchovy (an indeterminate-spawning pelagic roundfish, occupying much warmer southern waters) is not without merit. It is important to note, however, that significant differences could occur in the rate in which these species cycle between reproductive stages. Hunter and Macewicz (1985b, p. 87) caution that "the duration of postovulatory stages must be newly estimated for each species, and an assumption that the duration of these stages in a new species is similar to the northern anchovy is highly speculative." Perhaps further sampling of this arrowtooth population closer to or during the spawning season could have provided more information on the further development of these "spent or resting" fish.

Macroscopic classification was not successfully applied to the males. Only by histologic work was male maturity confidently assessed. Rickey (1995, p. 130), in her nearly year-round sampling of arrowtooth flounder, also had difficulty with assigning macroscopic maturity stages to males, stating that males ". . . did not show grossly apparent developmental changes over time . . . no spawning males were seen."

In the present study, females were classified as mature if they had oocytes as advanced as the cortical alveoli stage (Rickey, 1995), or showed atresia of previously vitellogenic oocytes. Significant differences in GSI and HSI occurred between the late perinucleus and cortical alveoli stages, and fish at the cortical alveoli stage were also longer and heavier. The insignificant but noticeable decline in condition factor in the cortical alveoli and early vitellogenic stages also indicates an emphasis in gonad growth over somatic growth. Rickey (1995), in examining Washington coast arrowtooth flounder collected during the spawning season, found that all of the fish had either matured beyond the cortical alveoli stage or had not yet matured that far. This finding supports the idea that fish in the cortical alveoli stage prior to the spawning season will mature during the same spawning season. Histology is regarded as the best method available to assess maturity (Hunter et al., 1992; West, 1990), but Hunter et al. (1992) concluded that even with the broadest criteria defining maturity, some spent fish are not identifiable as postspawners when sampling is done after spawning has commenced.

Hosie and Barss[1] determined that Oregon arrowtooth flounder males reach L_{50} at 29 cm and females reach L_{50} at 44 cm. Rickey (1995) determined that Washington males reach L_{50} at 28.0 cm and females reach L_{50} at 36.8 cm. Fargo et al. (1981) determined that British Columbia males reach L_{50} at 31 cm and females reach L_{50} at 37 cm. All the above studies determined maturity by using macroscopic classification of arrowtooth flounder gonads.

It was thought that macroscopic observations of maturity would result in a lower L_{50}, as all the other arrowtooth flounder maturity studies showed, but instead the L_{50} based on macroscopic observations was 3 cm higher. This finding is the opposite of that found in a study by Walsh and Bowering (1981) who compared macroscopic and histological staging of Greenland halibut (*Reinhardtius hippoglossoides*) ovaries and demonstrated that L_{50} was 3 cm higher in the maturity ogive derived from histological work.

Time of sampling in relation to the spawning season may have been a factor in determining female L_{50}. Hunter et al. (1992) showed that estimates of L_{50} for female Dover sole (*Microstomus pacificus*) taken during the spawning season were higher than estimates of L_{50} for female Dover sole taken just prior to the spawning season, whereas Rickey (1995) found

the opposite for arrowtooth flounder; her highest L_{50} values were derived from fish taken prior to spawning and her lowest value was derived from fish taken during the spawning season. The Oregon study occurred from September through June (Hosie and Barss[1]), the Washington study occurred nearly year-round (Rickey, 1995), and the British Columbia study occurred only in June (Fargo et al., 1981).

Histological examination revealed that most mature males in this study had only a small portion of their testes filled with spermatozoa; and thus they were not yet ready to spawn. It is likely that, as these large males continued to develop sexually during the season, other smaller males would have become sexually mature, thus lowering the male L_{50}. The male L_{50} value of 42.2 cm determined in this study should be viewed as a high estimate. Male GSI values started increasing at around 30 cm in length, and CF values began declining at 34.5 cm; both trends indicate a transition from somatic growth to gonad maturation at a much smaller size than that for the L_{50} reported here.

In general, the largest females were the most mature in this study, indicating that they might spawn the earliest. The high values of CF, GSI, and HSI for these largest, most mature females also show that these fish are best able to support the burden of spawning. The noticeable but insignificant drop in CF for females in the cortical alveoli and early vitellogenesis stages (Fig. 2), at around 50–60 cm in length (Fig. 3A), if real, can be explained by two possibilities. Either these mid-size fish are affected more by vitellogenesis than are the larger fish, or all females suffer losses in CF in the early stages of vitellogenesis and recover during later maturity stages. Gonadosomatic index was also highest in the largest males; thus they appear to mature earlier in the season than smaller males. The largest, most mature males had decreasing CF values that indicated an impact of maturing testes on body composition.

The spawning habits of arrowtooth flounder are not well known. Shuntov (1970) was unable to determine accurate spawning times for arrowtooth flounder in the eastern Bering Sea but nonetheless stated that they were close to those of Kamchatka flounder (*Atheresthes evermanni*), which were found in spawning condition in January and March. Fargo et al. (1981), using macroscopic observations of gonads collected in June from Hecate Strait, concluded that spawning takes place prior to June, probably in spring months. Rickey (1995) showed that spawning occurred off the Washington coast from September through December, and possibly as late as February. Hosie and Barss[1] reported a December–March spawning period for arrowtooth flounder off the Oregon coast. Pertseva-Ostroumova (1961) reported arrowtooth flounder spawning in the Bering Sea from January through March. The results presented here, that a spawning season begins after September, are supported by all of the studies mentioned above.

Total fecundity

Both macroscopic and microscopic observations showed that this study was made prior to the spawning season: none of the females were "ripe and running," had hydrated oocytes, or had postovulatory follicles, and none of the males were ready to spawn. Thus no bias due to loss of oocytes was expected.

Total fecundity estimates for arrowtooth flounder had not been previously reported in the literature. The only other member of the genus, Kamchatka flounder, has an estimated fecundity range of 130,000–500,000 oocytes (Pertseva-Ostroumova, 1961), which is much lower than what is reported here for arrowtooth flounder. As with many other flatfish species, arrowtooth flounder total fecundity increases linearly with fish weight and in a curvilinear fashion with length (Hempel, 1979). The largest arrowtooth flounder in this study had about 10 times as many oocytes as the smallest fish for which total fecundity was estimated. The unimodal frequency distribution of maturing oocyte diameters is supported by Rickey's (1995) determination that arrowtooth flounder is a group-synchronous spawner.

The significant difference in weight between the eyed-side and blind-side lobes has not been previously reported for arrowtooth flounder but has been reported for sole (*Solea solea*, Witthames and Walker, 1995). Nichol[2] found that blind-side lobes were significantly larger than eyed-side lobes in yellowfin sole (*Pleuronectes asper*). This finding suggests that in flatfish species both ovarian lobes should be considered when calculating GSI or total fecundity. Because there were no significant differences in mean oocyte diameter or mean oocyte density within or between ovarian lobes, total fecundity samples may be taken from any portion of the ovary. In his review paper, West (1990) mentioned that typically there is no difference in oocyte size or diameter frequency distribution between ovarian lobes, but differences along the length of the ovary and in cross sections do occur in some species. Hunter et al. (1992) found no differences in oocyte density between ovarian lobes, along the length of a lobe, or by cross section of a lobe in Dover sole.

[2] Nichol, D. G. 1995. Resource Assessment and Conservation Engineering Div., Alaska Fish. Sci. Center, 7600 Sand Point Way NE, Seattle, WA 98115. Personal commun.

Although this study provides the first information on total fecundity of arrowtooth flounders, subsampling of ovarian tissue for total fecundity, histology of males, comparison of macroscopic and histological methods, and relation of ancillary body measures such as CF, GSI, and HSI, there is still much that is unknown about this species. The lag of male maturity in comparison to that of females in this study, and the lack of spawning males in Rickey's (1995) study are parts of an interesting riddle. Possible seasonal migrations proposed by Shuntov (1970) and Rickey (1995) need to be thoroughly documented, as well as possible spawning migrations. Differences in size at maturity found in this study, compared with those found in studies conducted in southern waters, need to be explored. A study, in which collections are made at preselected depths and in which age and histological data are collected at regular intervals throughout a year, would answer many questions.

Acknowledgments

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The reproductive biology and early life stages of *Podothecus sachi* (Pisces: Agonidae)*

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Within the agonids, 20 genera and 50 species are recognized, with most distributed from the bottom of the north Pacific Ocean to the Bering Sea (Nelson, 1984). The agonid body is covered with bony plates and is unusual among teleost fishes. Several taxonomic studies have been conducted (Jordan and Evermann, 1898; Matsubara, 1955; Kanayama, 1991); however, little ecological information on the agonids exists because of their poor commercial value and small population density. Even for the sail-fin poacher, *Podothecus sachi*, the most common of the Japanese agonids, only larvae and juveniles have been reported from the adjacent waters of northern Japan (Maeda and Amaoka, 1988). Past reports concerning the reproductive ecology of agonids have suggested that they have internal fertilization and that females produce small clutches of eggs almost daily (Iioka and Gunji, 1979; Sugimoto, 1987; Aoyama and Onodera, 1989). Recently, eggs of copulating cottids that were thought to undergo internal fertilization were shown to be fertilized by the received spermatozoa only after eggs were deposited (Munehara et al., 1989, 1991, 1994a, in press; Koya et al., 1993), i.e. there is an internal deposition of sperm that do not penetrate the ova or egg until after the latter are spawned and free in the environment. This spawning mode, named the internal gametic association (Munehara et al., 1989), is characterized by the deposition of unfertilized eggs whose paternity has been fixed before spawning (Munehara et al., 1990; 1994b). This spawning mode is so unique that it is not included in other categories of the parity mode of fishes, as determined on an evolutionary basis (Wourms et al., 1988). A comparative osteological and myological study on Scorpaeniformes indicated that the Agonidae family is most closely related to the Cottidae family (Yabe, 1985). Therefore, it is possible that the internal gametic association mode of spawning that occurs in agonid fishes may provide information concerning the relation of the two families. In this study, I report on the reproductive biology and the early life stages of *P. sachi*, comparing them with those of other agonid fishes.

Materials and methods

Three adult females of *P. sachi* were collected with gill nets from offshore bottom waters (60–80 m depth) at Usujiri, southern Hokkaido, 7 October 1992. After the live fish had been transported to the laboratory, their ovaries were surgically removed, and ripe eggs and ovarian fluid were extracted. Great care was taken to prevent contamination by seawater, urine, and blood. The ovarian fluid was isolated with a pipette for use in the following test. To determine if egg development began before or after contact with seawater, eggs of each female were placed in separate petri dishes containing either ovarian fluid or seawater at 6°C. After 20 hours, the number of developing eggs were counted on the basis of occurrence of cleavage and formation of the blastodisc, which were regarded as signs of initial fertilization and autoactivation, respectively.

Histological observations were carried out on several eggs before exposure to seawater to determine if internal fertilization occurred. Ovaries were examined to decide their developing mode. The ovaries were fixed in Bouin's fluid. Serial paraffin sections, 5–8 µm, were prepared and stained with Delafield's hematoxylin and eosin. The criteria of the maturing oocytes followed the classification of Yamamoto (1956).

Eggs remaining from the above observations were used for morphological observation of the early life stages of this fish. The eggs not used for artificial insemination were kept in a 1-L glass dish at a mean water temperature of 5°C. No bubbling stone was placed in the dish, but half of the seawater was replaced once a week. Juveniles were fed nauplii of *Artemia salina*. Measurement and observation of embryonic development were conducted once every 1–3 days. Sampling of hatched fish was done at intervals of 1–2 weeks until 93 days after hatching. Specimens were observed under a microscope after fixation in 5% neutral formaldehyde solution. Terminology of the

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bony plates followed Gruchy (1969) and Maeda and Amaoka (1988).

Results

General anatomy and histology of the ovary

The paired ovary of *P. sachi* was bilobed anteriorly but fused together from the middle region; its posterior end reached beyond the genital duct, which was located at the middle of the abdominal cavity (Fig 1). The anterior part of the genital duct was retractable and could be everted by pressing the fish's belly. The protruded duct was tapered, about 2 cm length in 27.5–29.1 cm standard-length (SL) specimens (Fig. 1A). Blood vessels in the ovary ran radiately in the tunica of the dorsal side. The ovarian cavity passed through the center of the ovary and then directly faced the ovarian wall lined with epithelia near the genital duct. The genital pore opened just behind the pelvic fins. The cavity and the genital duct contained several hundred ripe eggs (Fig. 1B).

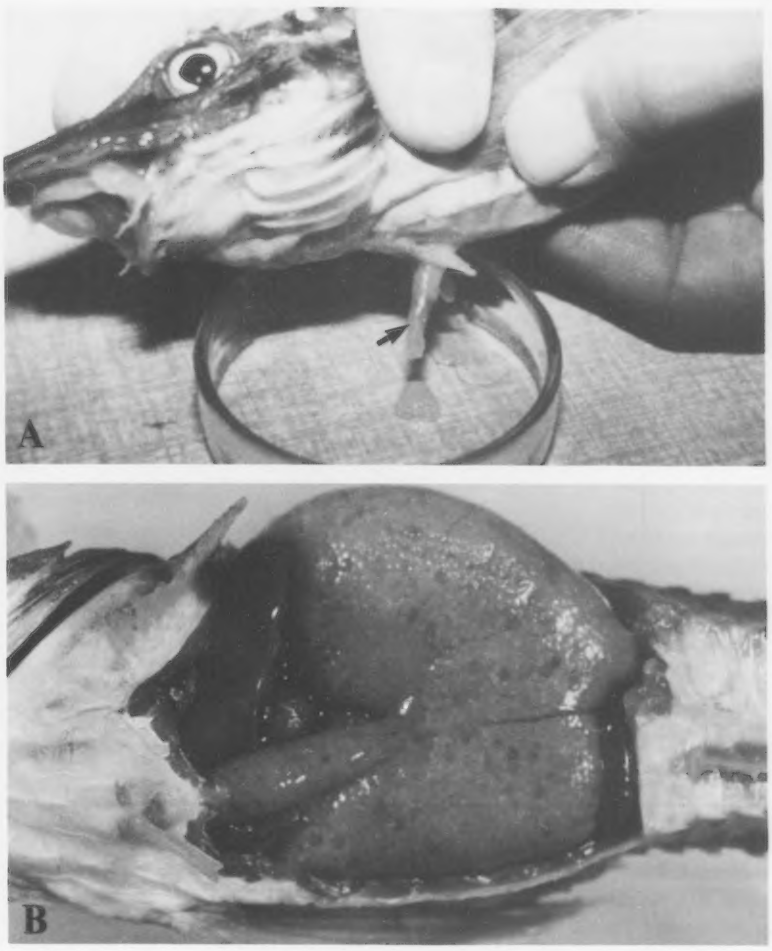

Figure 1

Retractable genital duct and mature ovary. (**A**) Genital duct (arrow) and eggs with ovarian fluid extracted from the top of the duct. (**B**) Ovulated eggs in the ovarian cavity and the genital duct.

Sections of the ovary contained oocytes in various developing stages, including the chromatin-nucleolus, perinucleolus, yolk-vesicle, oil-droplet, yolk-globule, migratory-nucleus, premature, and ripe stages (Fig. 2). In addition, postovulatory follicles in the stage just after ovulation or in the stage of regenerating were also found. These observations indicated that female *P. sachi* produce multiple clutches in a breeding season.

Appearance of eggs

Eggs were demersal, adhesive, and almost spherical in shape. The mean egg size was 1.73 mm in diameter, ranging from 1.70–1.75 mm (n=30). The yolk was light pink, and many oil droplets and small, whitish, granular material were observed within the yolk.

Initiation of egg development

Although the eggs from the three females were not artificially inseminated, most eggs placed in seawater had developed to the 2-cell stage after 20 hours (Table 1). In contrast, none of the eggs kept in the ovarian fluid showed any sign of development. When

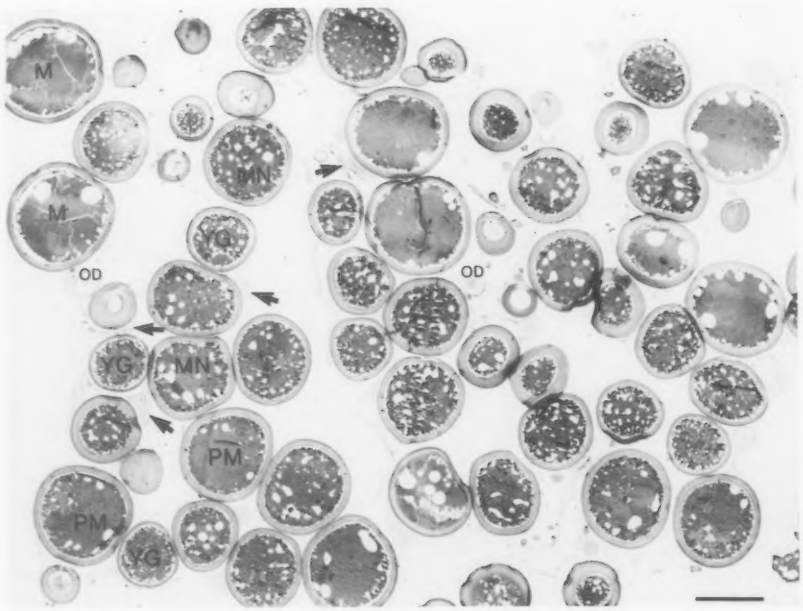

Figure 2

A section of mature ovary. M = mature stage oocytes, PM = premature stage oocytes, MN = migratory nucleus stage oocytes, YG = yolk globule stage oocytes, OD = oil droplet stage oocytes. Arrows indicate postovulatory follicles. Bar indicates 1mm.

Table 1

Embryonic development of *Podothecus sachi* eggs from 3 specimens 20 hours after immersion in seawater or ovarian fluid at 6°C.

Rearing medium	Percent (no.) of 2-cell stage eggs	Percent (no.) of undeveloped eggs	Percent (no.) of dead eggs
Seawater	97.5 (118)	1.7 (2)	0.8 (1)
Ovarian fluid	0 (0)	98.5 (133)	1.5 (2)
24 h after egg transfer from ovarian fluid to seawater	96.3 (130)	1.5 (2)	2.2 (3)
Seawater	95.5 (106)	3.6 (4)	0.9 (1)
Ovarian fluid	0 (0)	98.3 (117)	1.7 (2)
24 h after egg transfer from ovarian fluid to seawater	95.8 (114)	1.7 (2)	2.5 (3)
Seawater	97.2 (141)	1.4 (2)	1.4 (2)
Ovarian fluid	0 (0)	98.4 (124)	1.6 (2)
24 h after egg transfer from ovarian fluid to seawater	93.7 (118)	4.8 (6)	1.6 (2)

such eggs were transferred into seawater, they developed to the 2-cell stage within 24 hours. This finding indicates that egg development was initiated only after the eggs came in contact with seawater. It appeared that every female used in this study had copulated and that spermatozoa had already been transferred into the ovarian cavity.

Histological observation of gametes before exposure to seawater

The micropyle of *P. sachi* eggs consisted of a micropylar vestibule, a funnellike depression about 100 µm across at the level of the egg surface, and a micropylar canal penetrating the approximately 90-µm

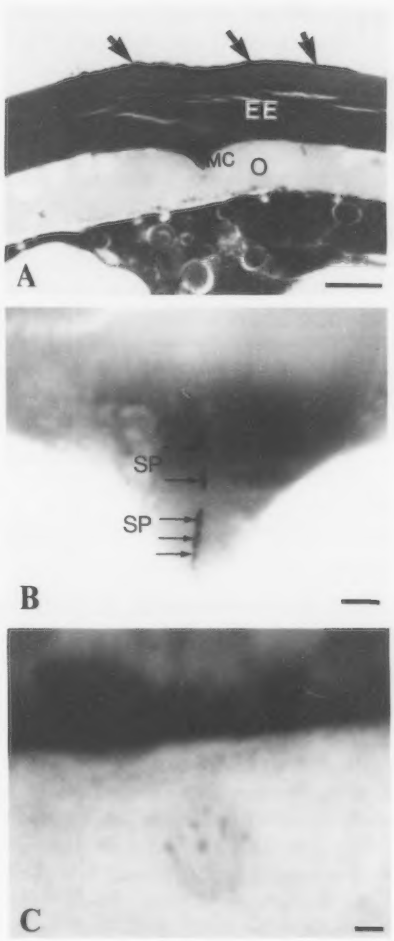

Figure 3

Photomicrograph of the eggs internally associated with spermatozoa. (**A**) Irregular particles (arrows) on and near the vestibule, and micropylar canal (MC). EE = egg envelope, O = ooplasm. (**B**) High magnification of micropylar cone in A. Spermatozoa (SP) have entered into micropylar canal. (**C**) Chromosomes and metaphase spindle of second meiotic division. Bars in A and B indicate 50 μm and bar in C indicates 10 μm.

thick egg envelope (Fig. 3A). The external opening of the canal was centrally located at the bottom of the vestibule. The canal was slightly tapered, and its inner opening was situated at the center of the micropylar cone. The external opening of the canal was about 5 μm in diameter. Many irregular particles stained with hematoxylin were deposited on and near the vestibule (Fig. 3A).

In eggs fixed before exposure to seawater, a number of spermatozoa were found to have entered the micropylar cone (Fig. 3B), but membrane fusion had not yet occurred. Furthermore, in the region of the ooplasm near the micropylar cone, chromosomes and a metaphase spindle of the second meiotic division were detected (Fig. 3C).

Embryonic development

After developing to the 2-cell stage after 20 hours of immersion in seawater, eggs reached the 32-cell, morula, and blastula stages on the 1st, 5th, and 8th days, respectively (Fig. 4A). The embryo became visible on the 13th day. The early embryo was smaller than the egg size, its length approximately 1/5 of the yolk's circumference. A pair of optic vesicles and optic lenses appeared on the 16th and the 21st day, respectively. Myomeres began forming on the 22nd day (Fig. 4B). On the 29th day, the tail of the embryo began to grow free from the yolk. The heart was pulsating and the embryo was moving occasionally on the 31st day. On the 35th day, a pair of otoliths was observed and the eyes began blackening. The embryo elongated to encircle the yolk completely by the 42nd day (Fig. 4C). A pair of pectoral fins began to extend at this time. Melanophores first appeared on the abdominal membrane on the 57th day. They began forming on the side of the trunk on the 62nd day. On the 76th day, the intestine and the liver were differentiated, and blood vessels appeared along the yolk below the thoracic region of the embryo. Just before hatching, the embryo measured 1.5 times the yolk circumference, and some projections of supralateral and infralateral bony plates were observed (Fig. 4D). Hatching began on the 92nd day and ended by the 104th day.

Larvae and juveniles

Newly hatched larvae were 6.9–7.1 mm in notochord length (NL) (Fig. 4E). Their bodies were slender, white, and pigmented on the head, trunk, and finfold. Yolks had not been completely absorbed yet, and an oil droplet remained in each yolk's anterior part. The larvae were weak swimmers and usually lay on the bottom of the tank. Most hatched 101 days after fertilization. On the 3rd day of hatching, not only supralateral and infralateral bony plates, but dorsal and ventral bony plates began to form. The larvae occasionally fed on *Artemia salina*. The urinary bladders of larvae were always swollen with transparent liquid. On the 14th day, the yolk was completely absorbed; a 7.4-mm-NL specimen had melanophores on the lateral sides of the abdomen and two pairs of fronto-

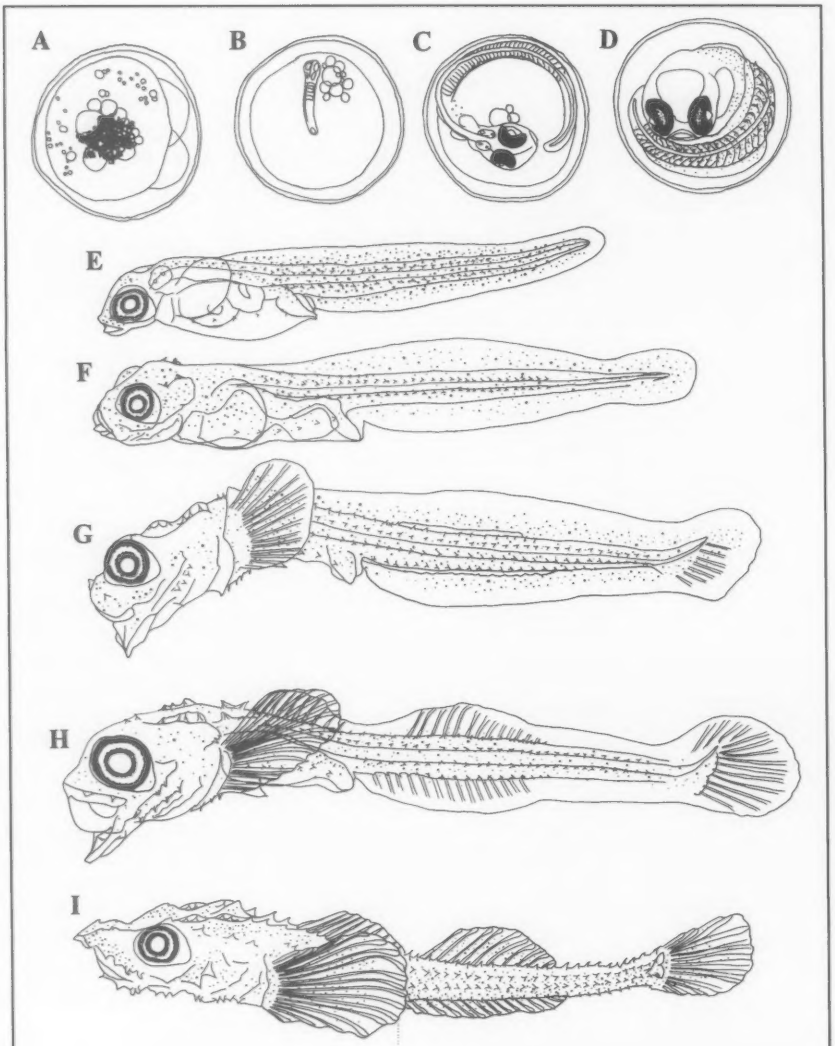

Figure 4

Eggs, larvae and juveniles of *Podothecus sachi* kept at 5°C. (**A**) 2-cell stage, 20 hours after immersion in seawater. (**B**) Formation of optic lenses, auditory vesicles, Kupffer's vesicle, and myomeres, 22 days after immersion in seawater. (**C**) Embryo almost in full circle, 42 days after immersion in seawater. (**D**) Just before hatching, 92 days after immersion in seawater. (**E**) Newly hatched larva 7.0 mm in notochord length (NL), 92 days after immersion in seawater. (**F**) 7.4-mm-NL larva, 14 days after hatching. (**G**) 7.7-mm-NL larva, 28 days after hatching. (**H**) Juvenile 8.7 mm in standard length (SL), 42 days after hatching. (**I**) Juvenile 15.6 mm in SL, 60 days after hatching. Dashed vertical line indicates the position of genital duct's opening.

parietal ridges with one spinule (Fig. 4F). The pectoral fins began to enlarge, and the larvae spent more time swimming near the surface than lying on the bottom. A 7.7-mm-NL specimen observed on the 28th day had 14 rays in its pectoral fins, melanophores on the ventral sides of the abdomen, and both jaws protruded slightly (Fig. 4G); four spines on preopercular and 2–4 spinules on each frontoparietal ridge were found. Another specimen measuring 8.3 mm NL on the 28th day was found to be in flexion; the larva

had 13, 16, and 15 rays in its dorsal, anal, and pectoral fins, respectively. On the 42nd day, a specimen measuring 8.7 mm SL had 10 spines and 13 rays on its dorsal fin and 15 and 16 rays on its anal and pectoral fins, respectively, forming a full complement (Fig. 4H). The pelvic fins were still buds. The opening position of the anal and the genital pore began moving from just anterior to the anal fin toward the base of the pelvic fins. Whiskers, a mustache, and pelvic fins first appeared in a 15.6-mm-SL specimen on the 60th day (Fig. 4I) and were completely formed in a 24.6-mm-SL specimen on the 93rd day. Juvenile *P. sachi* almost corresponded to adults in morphological features, but movement of the anal and genital pore openings were only half finished. The 24.6-mm specimen swam, using its elongated pectoral fins, but never used its posterior trunk for propulsion owing to hardened bony plates.

Discussion

Internal gametic association and external fertilization

As noted, *P. sachi* eggs placed in seawater and without artificial insemination began to develop and grow to juveniles, whereas eggs maintained in ovarian fluid showed no signs of development. In addition, histological observations of eggs directly obtained from the ovary showed that spermatozoa had entered the micropyle before contact with seawater, indicating that fertilization was not initiated in the ovarian fluid. This observation demonstrates that the spawning mode of *P. sachi* is of the internal gametic association type, reported in previous studies of copulating cottids (Munehara et al., 1989, 1991, 1994a; Koya et al., 1993). Atlantic wolffish, *Anarichas lupus* (Perciformes, Anarhichadidae), is also known to undergo copulation before egg laying, but the spawning mode of this fish is not comparable to that of *P. sachi* because inseminated eggs of Atlantic wolffish develop internally (Pavlov, 1994). This information seems to support Yabe's hypothesis (1985), based on comparative osteological and myological observations, that the family Agonidae may be the most closely related family to the Cottidae.

Early life history

Many larvae and juveniles, 8.3–25.1 mm SL, have been collected by plankton nets (Maeda and Amaoka, 1988). In the present study, the largest specimen took 93 days to grow to 24.6 mm SL after hatching. Thus, *P. sachi* probably inhabits the pelagic ocean during its first three months. It seems reasonable that whiskers and mustache, which function as sensory organs for detection of benthic prey (Sato, 1977), are completed before juveniles become benthic.

Reproductive style of agonid species

Information on the reproduction of agonids is available for only 6 of 50 species (Table 2). Many common characteristics are recognized among these species.

First, the reproductive behavior of the Agonidae involves copulation. All the agonid species whose reproductive styles are known (*Agonomalus proboscidalis*, *Occella iburia,* and *Bracdyopsis rostratus)* have been described as internally fertilizing species on the basis of the development of eggs deposited without male involvement (Iioka and Gunji, 1979; Sugimoto, 1987; Aoyama and Onodera, 1989). These agonids probably exhibit external fertilization with internal insemination, as does *P. sachi*, because *B. rostratus* has been determined to be of the internal gametic association type from the same investigations done for *P. sachi*, and information on internal fertilization of the agonids was proposed prior to the first recognition of the internal gametic association in teleost fishes (Munehara et al., 1989).

A second characteristic of the reproductive style is that agonids have a long embryonic period, ranging from 100 days to 1 year. In addition to the Agonidae, such extraordinarily long embryonic periods in teleost fishes are known for only a few trichodontids and cottid species (Okiyama, 1990; Munehara and Shimazaki, 1991).

The third and fourth characteristics are egg deposition in concealed sites and lack of parental care. Naturally deposited egg masses of *Agonus cataphractus* were collected from the roots of kelp (Breder and Rosen, 1966). Spawning of captive *Agonomalus mozinoi* and *A. proboscidalis* was performed by concealing eggs in the exoskeletons of invertebrates or between rocks (Marliave, 1978; Aoyama and Onodera, 1989). Egg masses of *B. rostratus* were found deposited on the bottom of a tank, but the spawner was kept in a bare tank with no suitable substrate (Sugimoto, 1987). It is still unknown where *P. sachi* spawns its eggs. However, it is probable that the spawning of this fish involves brood hiding and lack of parental care because this species has a retractable genital duct and a long incubating period, similar to other copulating cottids that deposit their eggs into sponges, polychaete tube colonies, and narrow fissures (Gomelyuk and Markevich, 1986; Munehara, 1991, 1992, 1996). Deposition of egg masses on such spawning substrates limits predation on the eggs. In addition, flagellar movements of

Table 2

Comparison of reproductive characteristics in some agonid fishes.

Species name	Spawning mode	Embryonic period and egg diameter	Spawning substrate	Parental care	Spawning system	References
Agonomalus mozinoi	unknown	unknown 1 mm	barnacle, tube worm[2]	without	remarkable iteroparity of small clutches	Marliave, 1978
A. proboscidalis	internal fertlization[1]	110–114 days 2.05–2.30 mm	between rocks and sand on bottom	without	remarkable iteroparity of small clutches	Iioka and Gunji, 1979 Aoyama and Onodera, 1989
Agonus cataphractus	unknown	1 year 1.76–2.23 mm	on roots of kelp	unknown	remarkable iteroparity of small clutches	Eherenbaum, 1936 in Breder and Rosen, 1966
Bracdyopsis rostaratus	internal gametic association[1]	287–324 days 2.1 mm	on bottom[2]	without	remarkable iteroparity of small clutches	Sugimoto, 1987; this study
Ocella iburia	internal fertilization[1]	unknown unknown	unknown	unknown	unknown	Sugimoto, 1987
Podothecus sachi[1]	internal gametic association[1]	92–104 days 1.70–1.75 mm	unknown	unknown	remarkable iteroparity of small clutches	this study

[1] The studies had been reported before publication of internal gametic association (Munehara et al., 1989).
[2] The findings were observed in aquaria.

host invertebrates, for aspiration and filtration, incidentally supply oxygen to eggs deposited inside or on the invertebrates (Munehara, 1991). The protracted period of incubation may have promoted egg deposition in any safe cradle for embryos rather than parental care.

Multiple clutches are produced by agonid species during a breeding season, as demonstrated by histological observation of the ovary of *P. sachi*; moreover, observations of *A. mozinoi*, *A. proboscidalis*, and *B. ostratus* indicate that females spawn small clutches almost daily in aquaria (Marliave, 1978; Iioka and Gunji, 1979; Sugimoto, 1987; Aoyama and Onodera, 1989). The fifth characteristic is the remarkable iteroparity of small clutches, which may have evolved in association with the laying of eggs, both into narrow spaces and without male involvement.

In summary, it is suggested that five common characteristics, i.e. copulation, a protracted period of incubation, concealment of deposited eggs, lack of parental care, and remarkable iteroparity of the reproductive ecology of agonids have been closely correlated with each other through evolutionary construction of their distinctive reproductive style. Copulation enabling impregnated females to spawn eggs without subsequent involvement of male fish seems to be a principal element of these characteristics.

Acknowledgments

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Age and growth of totoaba, *Totoaba macdonaldi* (Sciaenidae), in the upper Gulf of California

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The totoaba, *Totoaba macdonaldi* (Gilbert), also known as Mexican giant bass, is found only in the Gulf of California. This species during 1934–45 supported one of the most important sport and commercial fisheries in the Gulf, with total annual landings exceeding 2,000 metric tons (Rosales-Juárez and Ramírez-González[1]). At present, it is considered endangered (Flanagan and Hendrickson, 1976; NMFS[2]) as a result of 1) a high mortality of juveniles in shrimp trawl nets, 2) past overexploitation, 3) current illegal fishing during its reproductive season (early February to early May), and 4) ecological alterations of its spawning and nursery grounds. Flanagan and Hendrickson (1976) suggested that there was a high probability that this species would become extinct by 2000 AD. In 1975 the Mexican government declared a moratorium on fishing totoaba.

This paper reports on the age and growth of totoaba as determined from sectioned-otolith readings and contrasts current population age composition with what was known about the early population. Previous studies have reported ages based on nonvalidated scale readings (Nakashima, 1916; Berdegué, 1955; Molina et al.[3]).

Materials and methods

Totoaba were sampled in 1986–91 from the northern part of the upper Gulf of California between 31° and 32° N Lat. and 114° and 115° W Long. (Fig. 1). In 1989–91, juveniles were collected from shrimp trawl nets. Adults were sampled with gill nets during their reproductive season (Feb–Apr) of 1986, 1987, and 1989–91.

After determining individual standard (SL) and total length (TL) in millimeters and weight in grams, we extracted otolith (sagittal) pairs from 118 fish and embedded them in epoxy resin. For comparison with other age studies of totoaba, a linear regression was performed for converting total length into standard length. Lowerre-Barbieri et al. (1994) reported that sectioned otoliths were the best structure for ageing weakfish, *Cynoscion regalis*.

To permit data recovery when only severed heads were available, 118 otoliths were weighed (OW; +/– 0.001 g) and measured to determine their relation with SL (Pauly, 1984); only whole individuals were used in the present study. Maximum otolith length (OL; +/– 0.05 mm) was measured from rostrum to postrostrum margins (anterior–posterior), and maximum otolith thickness (OT; +/– 0.05 mm) from the dorsal to ventral margins (distal–proximal plane).

A transverse section was made from 101 otoliths with an Isomet low speed saw following a technique described by Beckman et al. (1990), Lowerre-Barbieri et al. (1994), and Secor et al.[4] and the otolith ring counts were read to determine age. Each thin section was read three times with transmitted light in a bright field by the same person. Following the criteria of Beamish and Fournier (1981), we calculated an index of average percent error for the single reader.

Three different axes (Fig. 2) were explored to measure the otolith radius (OR) of 94 thin sections. Annuli were most clearly counted and measured along axis 1; thus otolith radius (OR) was defined as the distance from the center of the core to the otolith outer edge along the ven-

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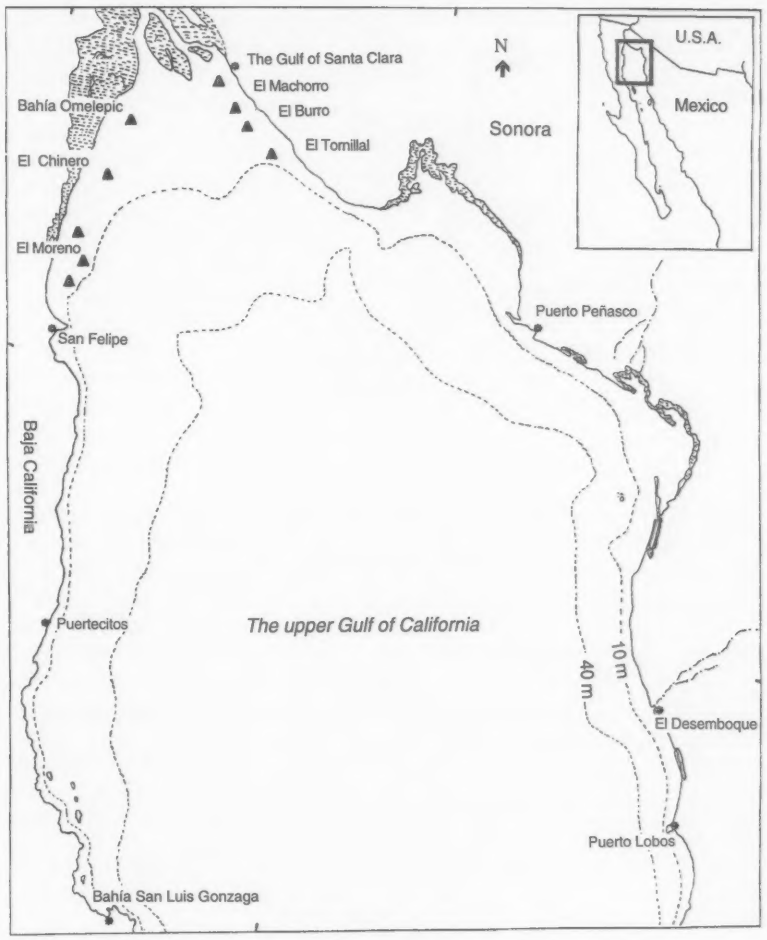

Figure 1

Location of sampling sites in the northern upper Gulf of California. (▲) = adults; juveniles were collected within the 40-m depth contour.

tral arm of the sulcal groove. The relation between otolith radius and fish length was fitted by using the Gompertz function (Ricker, 1979) and the computer program Fishparm 3.0 (Prager et al., 1989). To determine size at ages that were not collected, we backcalculated past ages following Bagenal and Tesch (1978) and Jerald (1983), using the Gompertz relation between OR and SL, not the otolith length to fish length regression.

A von Bertalanffy growth model (VBGM) was fitted to the observed SL at the midpoint of each age group represented in our sample (n=101) and also to the back-calculated sizes (n=346) determined from 81 otolith thin sections, by using the computer program Fishparm 3.0 (Prager et al., 1989). The growth equation was calculated for pooled sexes because juveniles can be sexed only by using histological techniques; age and length differences between males and females have not been reported in the literature.

Three juveniles captured in trawl nets during July and August 1989 were kept alive and transferred to the Centro Ecológico de Sonora Research Aquarium (Hermosillo, Sonora, Mexico); (see Almeida Paz et al. [1990] for more details). One fish died after almost 12 months of captivity; the other two were sacrificed 24 months after capture. Lengths and weights of these fish were taken every month beginning five months after capture. These lengths and the ring counts found in the otoliths of these fish were used to validate annual otolith ring deposition.

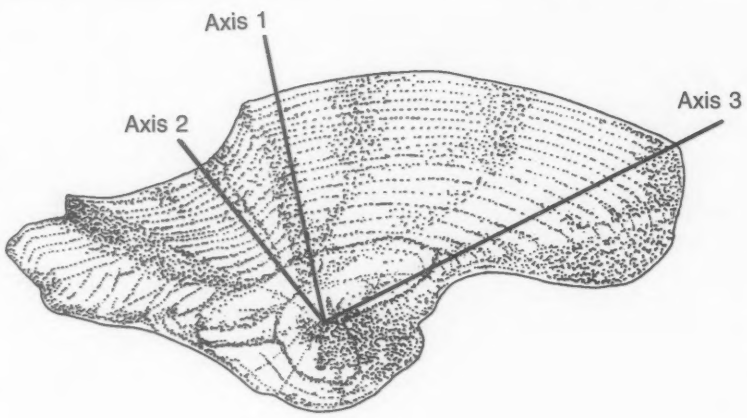

Figure 2

Three growth axes for radius measurements taken from otolith thin sections. Growth axis 1 data were related to standard length for back-calculation determinations.

Table 1

Meristic relations between totoaba standard length (SL) and total length (TL), otolith length (OL), otolith thickness (OT), and otolith weight (OW). For otolith measurements, n = 118.

Parameter	Equation	r^2
Fish total length (TL)	$SL = 0.91TL - 26.34$ (n=951)	0.99
Otolith length (OL)	$SL = 16.91 \exp(4.7(1-\exp(-0.96 \times OL)))$	0.99
Otolith thickness (OT)	$SL = 8.53 \exp(5.4(1-\exp(-0.17 \times OT)))$	0.98
Otolith weight (OW)	$SL = 788.8 \times OW^{0.4518}$	0.99

Results

From shrimp trawl nets, 1,125 juvenile totoaba (100–600 mm SL, mean=223, SD=65 mm SL) and 157 adults were collected (600–1,850 mm SL, mean =1,360, SD=89 mm SL). Figure 3 shows the length-frequency distribution for the specimens collected.

Totoaba sagittal otoliths are large and beanlike, as are most sciaenid otoliths (Secor et al.[4]). Table 1 shows the linear equation describing the relation between SL and TL, the Gompertz relations between SL and otolith length (OL), otolith thickness (OT), and the allometric equation for the SL and otolith weight (OW) relation (Fig. 4). Otolith growth in weight changes in relation to fish growth in length and is an allometric, not isometric relation (b=2.176, H_o: b =3.00, student's t-test $_{(0.025,\ 111)}$ =21.41).

Transverse sections of the totoaba otolith (Fig. 5) typically showed clear, opaque, and translucent zones and when the otolith is sectioned exactly through the focus, the core appears as a dense opaque zone, next to which the first annulus is found. The distance between the core and the first annulus is variable but is typically greater than increment sizes between the remaining annuli. The relation between otolith radius (OR) along radius 1 and SL (Fig. 6) was fitted to a Gompertz function:

$$SL = 30.92 \times \exp(3.86(1-\exp(0.99 \times OR))), \quad [r^2=0.98,\ n=94].$$

We found specimens representing 15 year classes between 0 and 24 years (Table 2). Of the 101 readable otolith sections, 66 fish were young-of-the-year (110–377 mm SL). Ten juveniles were of age class 2

(378–620 mm SL), and one was of age class 3 (740 mm SL). The remaining specimens were adults between age classes 4 and 24. The index of average percent error was 16.10% for the single reader.

Observed lengths and otolith ring counts were used to fit the von Bertalanffy model to obtain the growth curve for the totoaba population in the Gulf of California (Fig. 7); the fit was good (r^2=0.98, n=101). Past ages (n=346) were backcalculated from the thin-section otolith radius (OR) to fish length relation of 81 fish, and the von Bertalanffy growth model was also determined (Fig. 7). Table 2 compares the observed standard lengths with those calculated from both growth models. Figure 8 shows the relation between maximum whole otolith length and age as an exponential function.

In the case of the fish that died after a year of captivity (11 mo 21 d), its otoliths showed only one ring; the second fish held for two years, had two rings. The otoliths from the third specimen were decalcified and readings, unfortunately, were not possible. Fish held in captivity were captured in the same trawls as the rest of the juveniles used for age determination in this study. Otoliths of juveniles sacrificed at the time of capture did not present any rings or marks similar to those detected in otoliths of the individuals kept in captivity.

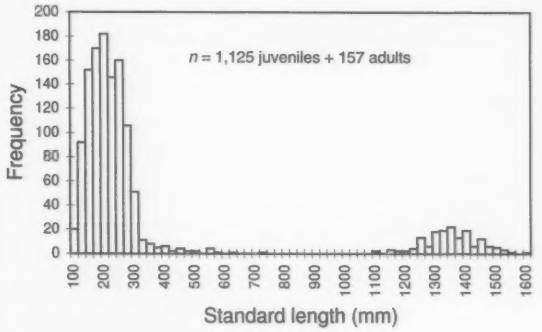

Figure 3

Length-frequency distribution for totoaba specimens collected in the Gulf of California. n = 1,282.

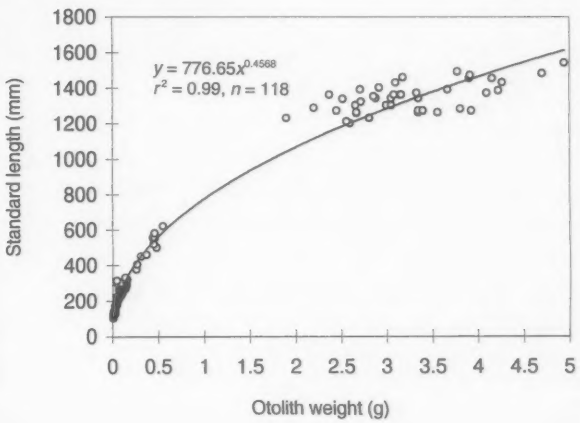

Figure 4

Relation between otolith weight and totoaba standard length, n = 118.

Discussion

The relation between otolith dimensions and fish length can be used to obtain data from the totoaba heads commonly found on the beaches of the northern Gulf. This relation is particularly important when one considers the restrictions and the potential impact of sampling an endangered species. Otolith growth and fish growth are proportional regardless of how growth rate changes with time; in early stages, both fish and otoliths increase faster than in adult stages after maturity is reached. Barbieri et al. (1994) reported for Atlantic croaker (*Micropogonias undulatus*) that age has an important effect on the otolith dimension to fish length relation. For totoaba, however, fish length alone described over 98% of the variability in otolith size. This growth pattern is common for other sciaenid species (Ross, 1988; Murphy and Taylor, 1989).

The relation between standard length and otolith radius in sciaenids has been fitted to several growth equations. Maceina et al. (1987) and Blake and Blake (1981) found good fit with a linear model, but Barger (1985) found the best fit with a power function. For totoaba in our study, the best fit was found with a Gompertz model for radius along axis 1. The wide range of radii at the maximum fish length (shown in Fig. 6), supports our assumption that annuli are formed throughout the life of the fish, as is also suggested by the power function fitted to the otolith length and age relationship (shown in Fig. 8). Barbieri et al. (1994) also reported the formation of annuli throughout the life of the Atlantic croaker. Although the otolith length to age relation could be used to estimate fish age, the fit is not as good as that between standard length and

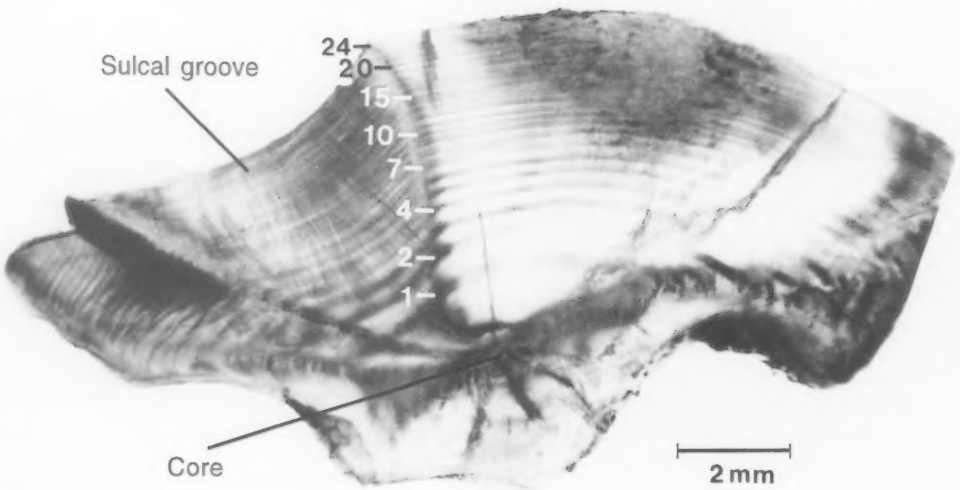

Figure 5
Photograph of transverse thin section of an adult totoaba otolith showing 24 annual rings, sulcal groove, and core.

age. No "Lee's phenomenon" (Ricker, 1969) was observed, and the von Bertalanffy growth model resulting from back-calculated data was very similar to that derived from observed data (Fig. 7 ; Table 2).

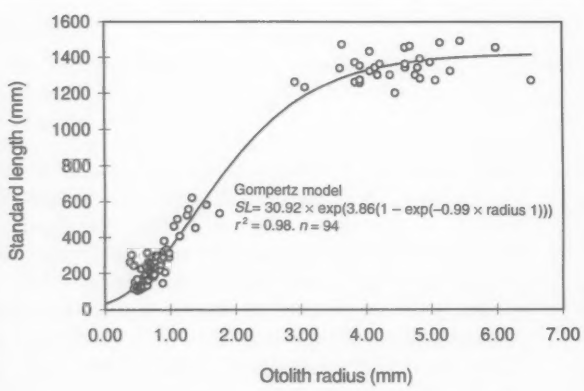

Figure 6
Gompertz curve used to fit the relation between totoaba standard length and otolith radius along ventral arm of sulcal groove (axis 1), $n = 94$.

Gauldie and Nelson (1990) commented that otolith growth is typically repressed on the ventral plane because this part of the otolith is in direct contact with the skull, which restricts otolith growth; although otolith growth ceases in the ventral plane, it continues to grow in the sulcular region. For totoaba, otolith growth seems to continue on the proximal side along the sulcal groove. The ventral arm of the sulcal groove has been reported as the best area for otolith reading in other sciaenids (Beckman et al., 1990; Lowerre-Barbieri et al., 1994).

Major increases in length occur during the first and second years, diminishing once totoaba reach their sixth or seventh year, the age at first maturity. After this stage, the growth curve reaches an asymptote from about the twelfth to fourteenth year. The observed adult mean standard lengths at age in our study are very close to those calculated by VBGM from observed data and also from back-calculated data.

In a comparison of age determinations with scales (Nakashima, 1916; Berdegué, 1955;

Table 2

Observed and predicted mean standard length at year-class midpoints. n = sample size. SD = standard deviation. VBGM = von Bertalanffy growth model. BC = back-calculated model.

Year class midpoint (yr)	n	Observed SL: mean (mm)	Observed SD	Predicted VBGM (mm)	Predicted VBGM BC (mm)
0.5	66	218	66	216	319[1]
1.5	10	503	75	525	595
2.5	1	740	—	750	797
3.5	1	1,080	—	914	945
4.5	0	—	—	1,034	1,053
5.5	0	—	—	1,121	1,133
6.5	0	—	—	1,184	1,192
7.5	1	1,260	—	1,231	1,234
8.5	0	—	—	1,264	1,266
9.5	0	—	—	1,289	1,289
10.5	1	1,271	—	1,307	1,306
11.5	0	—	—	1,320	1,318
12.5	2	1,363	38	1,329	1,327
13.5	5	1,318	88	1,336	1,334
14.5	4	1,309	102	1,341	1,339
15.5	5	1,340	24	1,345	1,342
16.5	1	1,390	—	1,348	1,345
17.5	1	1,300	—	1,350	1,347
18.5	0	—	—	1,351	1,348
19.5	1	1,280	—	1,352	1,349
20.5	0	—	—	1,353	1,350
21.5	1	1,490	—	1,354	1,350
22.5	0	—	—	1,354	1,351
23.5	0	—	—	1,354	1,351
24.5	1	1,453	—	1,354	1,351
Total	101				

[1] Otolith core to margin measurements along ventral arm of sulcal groove were used for year-class 0, and predicted lengths were derived from the resulting equation.

Flanagan, 1973; Molina et al.[1]), versus otoliths, as used in our study (Table 3), the greatest difference is found with Nakashima (1916). He estimated the maximum age for a fish of 1,980 mm (SL) to be nine years; our study shows that this fish could be older than 24 years. The von Bertalanffy parameters we estimated are very similar to those reported by Berdegué (1955) and Flanagan (1973). In the Molina et al.[1] study there was a large underestimation of maximum age in comparison with our results, reflected in the K value. This difference could be due to our use of a greater range of year classes from young-of-the-year to adults, whereas Molina et al.[1] used only adult fish. Juveniles or young-of-the-year should be included to fit the von Bertalanffy growth curve because if only adults are used, there is a tendency to obtain low K values (Beckman et al., 1990).

Scales often result in the underestimation of age (Beamish and McFarlane, 1987) owing to difficulty in reading, especially in the outer rings which are very close. Furthermore, there is a possibility of using regenerated scales in which the first rings that were formed are not included. Authors working with sciaenids have mentioned that reading scales is easier for short-lived species, like some species of *Cynoscion* (Villamar, 1972; De Vries and Chittenden, 1982).

On the basis of growth and otolith marks observed in two juveniles held in captivity, we suggest that ring formation in totoaba from the Gulf of California is annual; annual deposition patterns have been re-

ported for other sciaenids (Beckman et al. 1990, Murphy and Taylor 1991), including species of *Cynoscion*, a closely related genus (González, 1977; Blake and Blake, 1981; Shlossman and Chittenden, 1982; Barbieri et al., 1994; Lowerre-Barbieri et al., 1994). The well-defined seasonality in temperature in the Gulf of California (Alvarez Borrego et al., 1973; Paden et al., 1991) also suggests that the marks seen in totoaba otoliths are annual because such temperature changes are an important factor in ring deposition (Brothers, 1978; Beckman et al., 1990). Berdegué (1955) considered scale rings to be annual on the basis of the migratory pattern and reproductive period of totoaba.

The recent creation of the Upper Gulf of California and Colorado River Delta Biosphere Reserve will enhance conservation efforts for totoaba by protecting important spawning and nursery habitat. Furthermore, fishing pressure from commercial shrimp trawls and gill nets will be greatly decreased. Barrera-Guevara (1990) reported that 92% of young-of-the-year totoabas were killed in the commercial shrimp fishery. In our study we were not able to sample organisms between ages 5 and 11 because they were not available to trawls and gill nets and because we did not sample in areas where prerecruit totoaba concentrate. These areas are difficult to sample because of their depth; the Guaymas basin reaches more than 200 m depth. It has been suggested that the summer migration of totoaba is toward deep waters in the central Gulf of California (Berdegué, 1955; Arvizu and Chavez, 1972; Flanagan and Hendrickson, 1976). These fish, however, are accessible to hook and line fishing, and "catch and release" sport fishing practices should be encouraged.

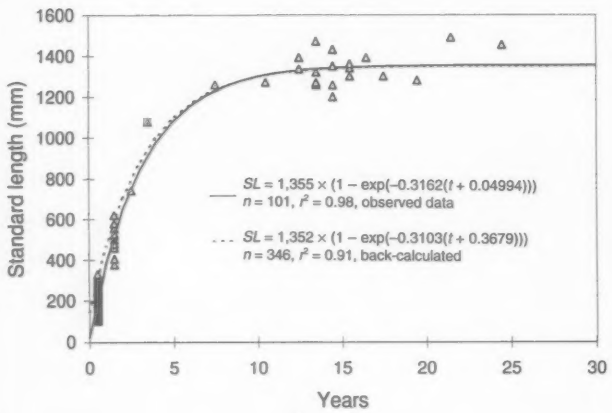
Standard length (mm)
Years
SL = 1,355 × (1 – exp(–0.3162(t + 0.04994)))
n = 101, r² = 0.98, observed data
SL = 1,352 × (1 – exp(–0.3103(t + 0.3679)))
n = 346, r² = 0.91, back-calculated

Figure 7

Von Bertalanffy growth model curve fitted to observed (___) and back-calculated (---) data of age (number of rings) and standard length (the year-class midpoint was used for age). Open triangles are observed data.

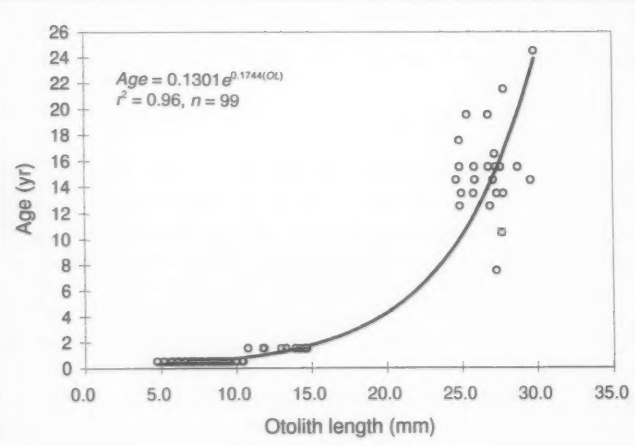

Figure 8

Exponential relation between maximum rostrum-postrostrum otolith length and age.

It is clear that the current available habitat for totoaba will not allow significant population increase. Nevertheless, we found a population age-structure similar to that existing during the early 1890's (assuming that fish of ages 3–11 years exist but were unavailable to our sampling as previously described), and we suggest that continued conservation efforts should allow for the survival of a stable but small population of totoaba in the Gulf of California. Estimates of adult survival proposed by Cisneros-Mata et al. (1995) before and after the 1975 moratorium also support evidence of stability in the current population age-structure.

Acknowledgments

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Table 3

Comparison of von Bertalanffy growth parameters for different studies of totoaba.

Author	K	L_∞	t_0	Max. age (yr)
Present observed data	0.3162	1,355	–0.0499	24
Present back-calculated data	0.3103	1,352	–0.3679	24
Nakashima (1916)	—	1,980	—	9
Berdegué (1955)	—	1,330	—	15
Flanagan (1973)	0.16	1,467	—	20
Molina et al. [1]	0.271	1,373	–2.264	19

[1] See Footnote 1 in the text.

gación Pesquera en Guaymas for assistance in the field with sampling juveniles. The Fish and Wildlife Foundation is acknowledged for support of field work during 1989. Sampling was carried out under Fisheries Research Permit No. 1229, Secretary of Fisheries, Mexico. We acknowledge the valuable suggestions of two anonymous reviewers.

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Mark retention and growth of jet-injected juvenile marine fish

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The type of mark or tag used for a particular study depends on the objectives of the study. Retention and recognition of a mark are critical to the success and reliability of a study. External tags have been the most common fish tags used (McFarlane et al., 1990), but they may affect survival, behavior, and growth of fish (Andersen and Bagge, 1963; McFarlane and Beamish, 1990). Each tag type has limitations and capabilities, but ideally external tags should be easily and rapidly applied to many fish, be inexpensive, easily identified, not easily lost or entangled, and not be stressful enough to alter fish survival, behavior, or growth. Studies that require such characteristics, therefore, are restricted in the type of tag that can be used and thus must rely on internal marks or dyes to identify fish. Identification of internal marks, however, generally requires that fish be killed, thus eliminating any possibility of repeated measurements of individual fish. Choice of mark is further restricted when marking juveniles that are of small size and that exhibit rapid growth.

Jet injection is a method of applying external marks to fish (Hart and Pitcher, 1969) that is relatively fast and can apply a variety of colors for either batch or individual marking. Jet injection does not affect survival or growth of juvenile salmonids in the laboratory (Cane, 1981; Herbinger et al., 1990; Thedinga and Johnson, 1995) but may contribute to increased mortality in field situations (Thedinga et al., 1994). Injection by Panjet has been used primarily on freshwater fish species (Hart and Pitcher, 1969) in addition to salmonids (Cane, 1981; Pauley and Troutt, 1988; Laufle et al., 1990). Juvenile flatfish have been marked with needle-injected latex (Riley, 1966) as well as by freeze branding (Dando and Ling, 1980), but not by jet injection. To our knowledge, juvenile sablefish have been marked only with Floy anchor tags (Rutecki and Meyers, 1992). Our objectives were to evaluate retention of jet-injected marks and their effect on growth of four marine fish species, as well as their effect on the tissue structure of three marine species held in the laboratory.

Methods

We tested the retention of jet-injected marks and effects of marks on growth of four species of marine fish and histological effects on three species of marine flatfish. We injected two substances into juvenile sablefish, *Anoplopoma fimbria*, and one substance into juvenile yellowfin sole, *Pleuronectes asper*, rock sole, *Pleuronectes bilineatus*, and Pacific halibut, *Hippoglossus stenolepis*. Sablefish were captured by hand-jigging in St. John Baptist Bay near Sitka, Alaska, September 1993 (Rutecki and Meyers, 1992). Sole were captured by beach seining in Auke Bay, Alaska, May to July 1994, and halibut were collected by trawling in Sitkinak Strait and Ugak Bay near Kodiak Island, Alaska, August 1994.

A total of 28 sablefish and 30 flatfish were injected with a Panjet in 1993–94. Sablefish were marked with alcian blue dye (65 mg/mL aqueous solution) and fluorescent orange acrylic paint (Liquitex, 50% aqueous solution); 10 of each flatfish species were marked with alcian blue dye. All sablefish were marked on the abdomen between the pelvic fins (Fig. 1). Flatfish, however, were marked with individual identifying marks on the ventral surface at one to four locations along the lateral margin and on the caudal peduncle (Fig. 2); 12 sablefish and 10 of each flatfish species were left unmarked as controls.

The Panjet was held about 25 mm from the fish's skin during marking. Sablefish were anesthetized with tricaine methanesulfonate (MS-222) before marking but flatfish were not. After marking, excess dye or paint was rinsed off with water to check mark quality. If a mark was good, the fish was put in a recovery tank; if poor, the fish was remarked.

Sablefish and flatfish were held in different environments. After being marked, sablefish were held in 600-L flow-through tanks for 238 days. Because of space restrictions, blue-marked and control fish were held in one tank, but each group was kept in separate compartments. Orange-marked fish were held in another tank but died prematurely in a laboratory accident

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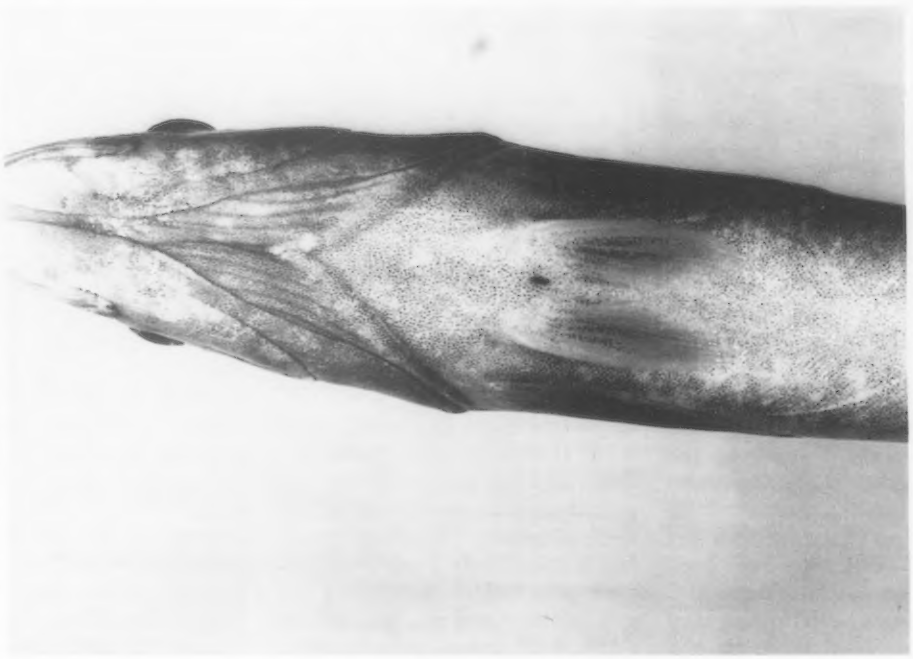

Figure 1
Panjet-injected alcian blue dye mark between the pelvic fins of a juvenile sablefish.

after 189 days and were frozen prior to analysis. Flatfish were held for 90 d in six 70-L flow-through tanks on their preferred bottom type (mud substrate for soles [Moles and Norcross, 1995], sand substrate for halibut). For each flatfish species, control and marked fish were held in separate tanks. Sablefish were held indoors and were provided about 8 h of fluorescent light daily, whereas flatfish were held outdoors under an awning with 12 h of fluorescent light daily. Sablefish were fed ad libitum, and flatfish were fed 10% of their initial body weight per day throughout the study. The substrates in the flatfish tanks were initially frozen three days to kill meiofauna and macrofauna. Mark recognition for sablefish was checked about every 3 weeks, flatfish about every 4 weeks. Blue marks were viewed under fluorescent light, orange marks under fluorescent and ultraviolet (UV) light. Mark retention was rated subjectively as acceptable (retained) or unacceptable (not retained) by the same person each time the fish were checked. Fish lengths were recorded at the beginning and end of the study: fork length (FL) for sablefish, total length (TL) for flatfish. Because we were obtaining additional data (histological) from flatfish, we also recorded flatfish weights when we recorded their lengths. Differences in acceptable mark retention were tested with a chi-square test, and differences in fish size and growth rate were tested with a t-test.

Absolute growth rate in length of flatfish was calculated as

$$L = TL_2 - (TL_1/90)(10),$$

where L = absolute growth in length;
TL_2 = total length at 90 days; and
TL_1 = total length at day 1 (beginning of the study).

Instantaneous growth rate in weight of flatfish was calculated as

$$W = (\log_e W_2 - \log_e W_1)/90,$$

where W = instantaneous rate of increase in weight;
W_2 = weight at 90 days; and
W_1 = weight at day 1 (beginning of the study).

All flatfish were examined for histological changes. Fish were examined for gross pathology at 50× with a dissecting microscope. Gill and liver tissues were

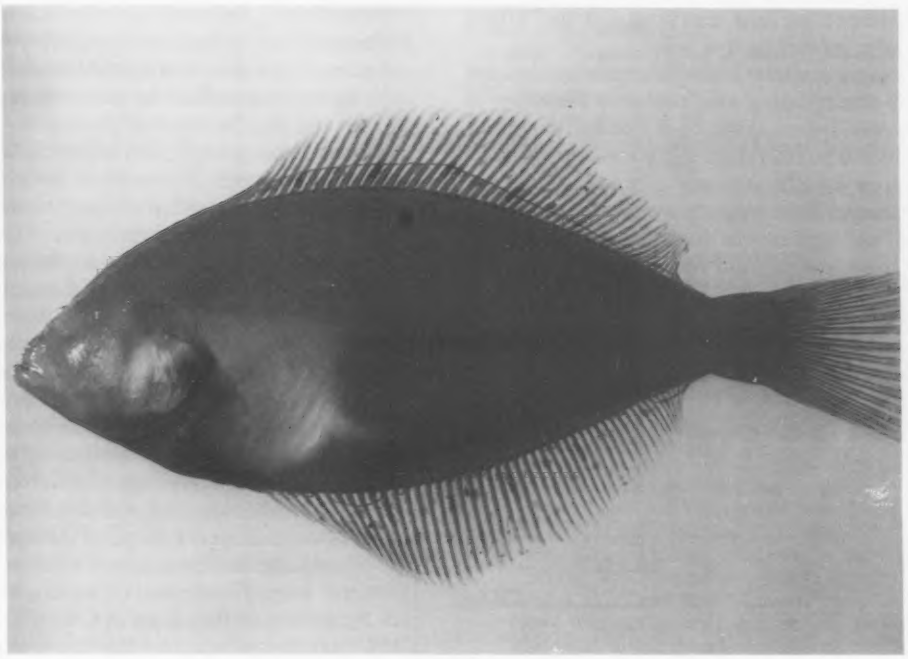

Figure 2
Panjet-injected alcian blue dye mark on the ventral lateral margin of a yellowfin sole.

excised and fixed in 10% buffered seawater. Tissues were then placed in 70% ethanol for two days, dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin, and sectioned at 6 μ. Sections were stained with hematoxylin and eosin and examined for lesions and evidence of wound healing.

Results

For sablefish, mark retention varied with mark color and method of detection. Retention of marks was significantly higher ($P<0.001$) for alcian blue dye (100%) than for fluorescent orange acrylic paint when orange marks were viewed under fluorescent light but was similar ($P=0.99$) when orange marks were viewed under UV light (Fig. 3). Retention of orange marks viewed under fluorescent light was 100% after 21 days but decreased to less than 20% after 84 days. Retention of orange marks viewed under UV light, however, decreased only to 92% after 84 days and remained at that level throughout the remainder of the study.

Mean length at the end of the study was similar ($P=0.24$) between blue-marked sablefish (280 mm) and controls (288 mm) (Table 1). Despite the potential for tank-linked effect due to holding the marked fish in a separate tank, mean length of the orange-marked sablefish (270 mm) was not significantly different from that of the controls (288 mm) ($P=0.06$) (Table 1). Mortality was zero.

Table 1
Mean fork length of jet-injected and control juvenile sablefish at time of injection and after 33 weeks. Fish were injected with alcian blue dye and fluorescent orange acrylic paint. Standard error is in parentheses.

	Mean fork length (mm)	
	Initial	After 33 weeks
Blue	212 (0.80)	280 (1.07)
Orange	212 (0.96)	270 (1.18)
Control	218 (1.16)	288 (1.48)

For all flatfish, mark retention was 100% throughout the study, and growth was similar between marked and control fish (Table 2). The instantaneous rate of increase in length and weight at the end of

the study was similar ($P \geq 0.10$) for marked and control fish, indicating that marking did not affect growth. Again, mortality was zero.

Histological examination of flatfish indicated that the marks were nonirritating and nontoxic. Necropsy of the flatfish revealed no evidence of damage to skin or musculature and no alteration in the structure of dyed tissue. All liver hepatocytes were normal, indicating no toxic exposure. There was no evidence of increased macrophage aggregations in the liver or hyperplasia in the gills to suggest cellular responses to dyes.

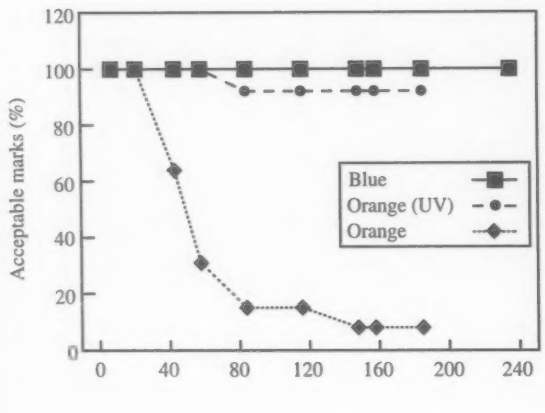

Figure 3

Percentage of acceptable jet-injected marks on juvenile sablefish by color: alcian blue dye and fluorescent orange acrylic paint. Dashed line is orange marks viewed under ultraviolet light (UV), and dotted line is orange marks viewed under fluorescent light.

Discussion

Marine fish can be jet injected rapidly with many individual marks, often without anesthesia. For example, in this study we marked several nonanesthetized flatfish per minute. Jet-injected alcian blue dye produced a highly visible intradermal mark that was retained for at least 8 months by juvenile sablefish, 3 months by juvenile flatfish. Detection of alcian blue dye marks on flatfish is easy and requires minimal handling. Usually marks can be detected without anesthesia and without turning fish over. Increased visibility of jet-injected marks, however, could make fish more conspicuous to predators. Fluorescent orange acrylic paint marks, however, faded rapidly, making marks less visible to predators but necessitating the use of UV light for detection.

Mark retention was similar to that reported for other species. Thedinga and Johnson (1995) reported 96% retention of alcian blue dye and fluorescent orange acrylic paint marks on the caudal fin of juvenile coho salmon, *Oncorhynchus kisutch*, and sockeye salmon, *O. nerka*, after nearly 4 months, and Herbinger et al. (1990) reported 96% retention of alcian blue dye-marked Atlantic salmon, *Salmo salar*, after 6 months. Few studies have been published that used marked juvenile sablefish or flatfish, and only one used a dye mark. Kelly (1967) injected Fast Blue 8GXM and hydrated chromium oxide by needle into the heads of juvenile winter flounder, *Pleuronectes americanus*, and had 100% retention after 4 months.

Jet-injected marks did not affect growth or mortality. Unlike Petersen disc and roll tags, which depressed growth rates (Andersen and

Table 2

Mean initial total length (mm) and weight (g) and mean absolute growth rate in length and instantaneous growth rate in weight of marked (jet-injected with alcian blue dye) and control juvenile yellowfin sole, rock sole, and halibut 90 d after marking. Standard error is in parentheses.

	Initial size		Growth rate after 90 d	
	Marked	Control	Marked	Control
Length				
Yellowfin sole	67.9 (1.04)	74.2 (1.45)	0.198 (0.067)	0.196 (0.054)
Rock sole	59.7 (0.92)	71.6 (1.56)	0.161 (0.068)	0.137 (0.073)
Halibut	72.3 (0.81)	71.8 (0.88)	0.228 (0.059)	0.241 (0.050)
Weight				
Yellowfin sole	3.5 (0.49)	5.2 (0.64)	0.823 (0.144)	0.841 (0.120)
Rock sole	2.3 (0.32)	5.2 (0.68)	0.610 (0.171)	0.817 (0.153)
Halibut	4.1 (0.36)	4.1 (0.36)	1.193 (0.109)	1.175 (0.111)

Bagge, 1963) and resulted in increased mortality in sablefish (McFarlane and Beamish, 1990), jet-injected marks did not affect flatfish and sablefish growth or survival. Marks, however, may not be retained as long under natural conditions where growth is faster: sablefish in their natural habitat average 31–33 cm FL in spring (McFarlane and Beamish, 1983) in contrast with 28 cm FL recorded at the end of our laboratory study in spring.

Jet-injected marks did not affect fish histology. There was no evidence of lesions in skin or musculature and no alterations in either the cells or the structure of dyed tissues. Changes in liver hepatocytes occur when fish have been exposed to toxicants (Hinton et al., 1992) but test hepatocytes in our preparations were normal.

Jet-injection of either alcian blue dye or fluorescent orange acrylic paint is a good method for mass marking or individually marking juvenile marine fish and meets most criteria for an effective external marker (Kelly, 1967). The marks are effectively retained and nontoxic and nonirritating; they do not affect mortality, can be used rapidly, and are inexpensive, readily visible, and permit numerous different mark combinations (Thedinga et al., 1994). Their application requires minimal training and equipment. Most importantly, jet injection does not alter the growth or tissue structure of fish. A limitation of jet-injection marking is the nonpermanent nature of the mark (Thedinga and Johnson, 1995). As a moderate-lasting marking method of juvenile marine fish, it is superior to most available external marking methods.

Acknowledgments

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Erratum

Erratum: Fishery Bulletin 95(1):11–24.
Crockford, Susan J.
Archeological evidence of large northern bluefin tuna, *Thunnus thynnus*, in coastal waters of British Columbia and northern Washington

Correction: Susan Crockford has brought to our attention an error in the last column of data in her original Table 4 (page 18). The correct data for estimated FL (cm) should read as follows:

Table 4

Archeological bluefin tuna vertebrae measurements and fork length estimates, by vertebrae number. All specimens. Measurements are defined in Figure 2.

Vertebra no.	Centrum GL (mm)	Centrum GB(p) (mm)	Estimated FL (cm)	Vertebra no.	Centrum GL (mm)	Centrum GB(p) (mm)	Estimated FL (cm)
01	27.0	44.4	198.7	21	38.3	45.1	189.6
02	26.3	48.2	191.5	22	39.2	44.8	188.6
04	22.5	39.6	164.7	22	39.2	46.5	193.5
04	31.6	65.1	224.9	24	39.4	47.5	195.3
05	27.1	58.7	212.4	24	45.1		209.8
06	30.8	58.9	218.2	25	40.8	48.3	194.8
09	25.6	34.4	168.2	26	40.1	47.6	190.7
(09)		48.7	220.8	(28)	30.2	35.9	159.3
(09)	22.3	30.5	153.6	29	33.7	36.3	155.3
(10)	33.4	45.0	206.7	29	45.3	49.5	201.1
11	24.4	34.6	165.5	29	50.3	58.2	221.1
(11)	31.0	37.8	177.5	30	28.1	32.0	138.5
(12)	35.0		200.5	30	36.7		167.1
(12)	36.7	46.5	206.9	30	38.3	44.0	172.3
(12)	34.9	41.9	192.0	30	46.7		199.2
14	33.5		187.0	30	47.3	50.0	201.1
(14)	33.9	40.9	185.4	30	47.5	51.2	201.7
(14)	34.1	42.0	189.3	31	48.6	52.8	201.3
(14)	32.5	42.1	189.7	31	52.7	53.8	215.3
(14)	27.7	32.8	157.8	32	41.7	45.6	179.3
15	35.4	42.5	188.4	32	43.7		185.6
(15)	31.4	42.5	188.4	32	49.7	54.4	204.4
16	35.9	45.6	191.2	33	26.6	27.0	122.4
16	36.1	42.9	191.9	33	43.8	44.8	178.9
(16)	31.3	35.4	175.0	33	44.5	47.2	186.5
(16)	33.8	40.9	183.9	33	44.7	45.6	181.4
(16)	40.2	47.2	206.0	33	47.9	53.9	207.3
(16)	41.2	51.8	209.4	33	52.2	53.7	206.7
17	36.0	42.8	187.3	33	53.7	55.2	211.3
17	37.5	47.3	200.2	34	48.1		202.4
(17)	37.0	43.9	190.4	34	48.2		202.6
(17)	38.2	44.2	191.3	35	41.2	42.0	200.8
18	37.2	46.5	195.4	36	30.5		198.9
(18)	26.8	32.4	153.5	38	11.9	28.2	210.3
19	39.4	47.2	196.0	38	16.5	31.8	243.2
(19)	27.2	32.7	151.9	39		23.0	198.3
20	35.5	44.5	182.4	39		19.5	174.3
(20)	38.0	45.0	191.3				
(20)	44.3	54.0	213.4				

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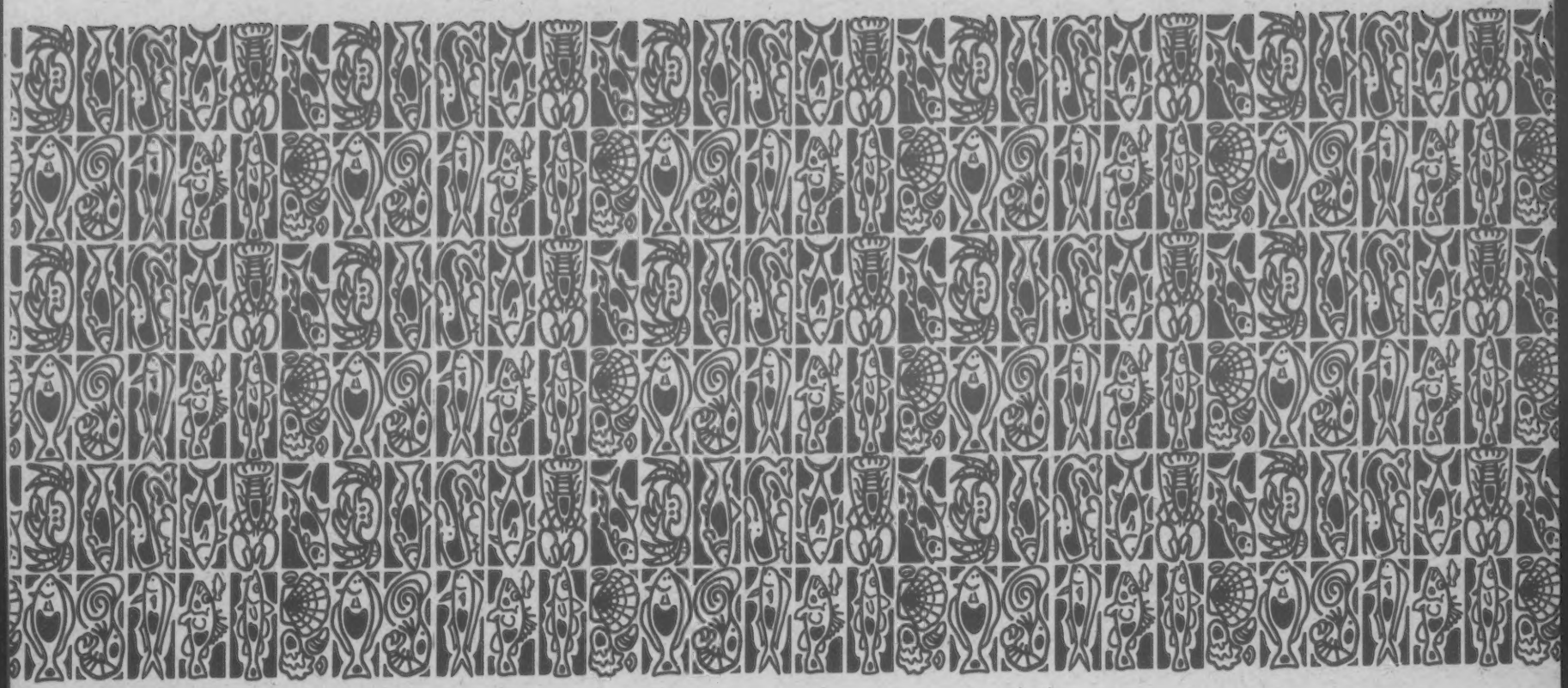
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